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Book of Abstracts

61st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Date/Location:	**1. – 5. September 2013, Münster, Germany**
Chairmen:	Andreas Hensel, Thomas J. Schmidt
Issue Editors:	Andreas Hensel, Matthias Lechtenberg, Thomas J. Schmidt

Dear Colleagues,

The Society for Medicinal Plant and Natural Product Research (GA) invites scientists from academia and industry dealing with all aspects of medicinal plant and natural product research as well as phytotherapy to its 61st International Congress and Annual Meeting. This important scientific meeting will be held in Münster, a picturesque city surrounded by beautiful countryside in the heart of Westphalia, in the Northwest of Germany. Münster once played an outstanding role in history. The peace treaty of Münster was signed here as part of the "Westphalian Peace" of 1648, which ended the Thirty Years War and paved the way to a modern Europe as we know it. Today, Münster is a lively young city with the University "Westfälische Wilhelms-Universität" (WWU) as a major employer and regional center of research and education. With more than 40.000 students and an exciting research profile WWU is one of the biggest and leading universities in Germany. The conference will take place in the central university area, in front of the historic castle of Münster which is located in the middle of the city's historic downtown area.

Our scientific programme has attracted the interest of a true host of researchers worldwide so that we look forward to welcoming more than 600 scientists from over 60 countries. The main topics of the congress are:

- Natural products against neglected diseases
- *In vivo* phytopharmacology
- Skin-active natural products
- Glycobiology and carbohydrate-based active compounds
- Ethnopharmacology of African medicine
- Ethnopharmacology of Amazonian medicine
- Computational methods in natural products chemistry
- Hyphenated analytical techniques and target fishing
- Natural product chemistry
- Plant phenols: structures, analytics and activities
- Quality control methods for medicinal plants, extracts and isolated natural products
- Herbal medicinal products in animal healthcare and veterinary medicine

The program is highlighted by nine invited lectures delivered by distinguished speakers covering the major scientific topics of the conference. More than 75 short lectures will present the current state of the art in natural product science, and especially younger scientists, but also researchers from industry will present high quality results for intense discussion. Workshops dedicated to topics of particular interest will provide in-depth insights into various aspects of medicinal plant and natural products research.

Three poster sessions, scheduled within central time frames during the congress, will bring together scientists from different disciplines and stimulate the exchange of experiences and knowledge.

It is a pleasure to the organizers that the European Cooperation in Science and Technology within the COST 1006 framework will be integrated into the meeting by a presymposium "*Challenges and Limitations in Metabolic Pathway Engineering of Secondary Natural Products*" highlighting the latest insights in biotechnical production of natural products for medicinal applications.

In addition to presentation and discussion of excellent research it will be a major goal of this conference to provide a basis for intense networking between scientists of academia and industry from all over the world.

The present issue of *Planta Medica* devoted to the congress was realized by the efficient and excellent work of Dr. Matthias Lechtenberg and by many staff members from the Institute of Pharmaceutical Biology and Phytochemistry. We express our deepest gratitude to all of them for helping us to publish high quality abstracts in this volume. We also thank the many members of the scientific committee, for their meticulous help in evaluating more than 700 abstracts and – last but not least – the many dedicated researchers who submitted so much of their excellent work!

We wish you all a pleasant and fruitful meeting with science, culture and hospitality!

Andreas Hensel and Thomas J. Schmidt

Lectures

L1 [Opening Lecture] Natural products and drug discovery for tropical diseases – successes, failures and lessons learned

Fairlamb AH
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Infectious diseases of poverty are responsible for countless deaths and enormous disability in tropical and sub-tropical regions of the planet. Current treatments include natural products and synthetic drugs, but many of these are unsatisfactory for reasons such as toxicity, poor efficacy and safety, the need for hospitalisation, lack of oral bioavailability, drug resistance and high cost. Lack of investment due to poor economic return has been a major disincentive for the pharmaceutical industry to venture into drug discovery for neglected diseases. To try to overcome this obstacle, several academic groups are now engaged in early stage drug discovery (up to preclinical candidate) in order to maximise the chances of success by de-risking discovery projects. Some of the successes, failures and lessons learned by the Drug Discovery Unit and by my own laboratory will be presented, with examples from phenotypic- and target-based projects in malaria, sleeping sickness, visceral leishmaniasis and Chagas' disease.

L2 [Plenary Lecture] Ethnopharmacology examples from Amazonian folk medicine

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Ethnopharmacology is the discipline that focuses in the understanding of human interactions with plants in the context of health care. Interest in sources of prototypic drugs includes plant derived compounds, and thus leads derived from ethnopharmacology. Ethnopharmacological analysis of traditionally used plants can not only point to promising species but also generate laboratory hypothesis on which therapeutic properties might be expected, a decisive advantage to select the *in vitro* and *in vivo* models to be employed in order to identify and characterize pharmacological properties. Valuing traditional knowledge and biodiversity in the context of pharmaceutical innovation is therefore of particular interest to countries that are rich in sociobiodiversity, such as Brazil. Folk medicine in Brazil is often described as a rich mixture of African, European and Amerindian medical traditions. Nevertheless, this is a rather simplistic view given the complex exchange during colonial times and the mosaic of influences from the nearly 1.5 million immigrants from a diversity of cultures that came to Brazil between the 19th and 20th centuries. Inasmuch as Amazonian medicine is heavily influenced by each of the local dominant indigenous groups (among the more than 234 ethnically distinct native peoples that originally inhibited Brazil) in specific sub regions, what is currently understood as the Amazonian folk medicine is best expressed among caboclos (riberinhos or Amazonian peasants). Three examples of ethnopharmacology oriented research, namely the analgesic alkaloids from *Psychotria colorata*, the hypnosedative monoterpene from *Aeollanthus suaveolens* and the neuroprotective properties of *Ptychopetalum olacoides* will be used to illustrate Brazilian Amazon native remedies as well as the rationale and usefulness of ethnopharmacology in the search for new drugs from plant origin. Suported by CNPq.

L3 [Plenary Lecture] African Ethnopharmacology: The Southern African Perspective

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Southern Africa is endowed with a rich floral biodiversity and cultural values of which the inhabitants have taken full advantage. The importance of traditional healers and remedies made from indigenous plants play a crucial role in the health of millions, making traditional herbal medicines an important part of the healthcare systems. This impressive biodiversity, however, presents researchers with many challenges, opportunities and responsibilities. This presentation describes the role southern Africa has played in contributing to the worldwide increase of knowledge in the field of ethnopharmacology. Emphasis will be placed on the screening of medicinal plants in southern African for ethnopharmacological properties which primarily focus on investigating the efficacy of these plants in the search for new therapeutic products. Other important aspects of medicinal plant research related to safety of indigenous medicinal plants and the conservation and sustainable use will be highlighted. Issues on the preservation of indigenous knowledge systems will be tackled. Emerging research areas such as infectious and neglected diseases such as tuberculosis and venereal diseases will be placed under the spotlight. In addition to ethnopharmacological aspects of southern African traditional medicine, issues pertaining to the development and improvement of traditional medicinal plant research are equally important. These include the successful commercialisation of key medicinal plants, legislation to regulate trade and projections on research funding. It is hoped that this presentation will draw attention to the great opportunities for improved research on southern Africa's rich floral biodiversity and cultural values towards the development of herbal medicines through sustainable utilization of the floral heritage.

L4 [Plenary Lecture] Current state and future perspectives of natural products-based drug discovery in Africa

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Investigating the virtually untapped African natural resources of novel molecules remains a major challenge and a source of novelty in the era of combinatorial chemistry and genomics. Nevertheless, Africa biodiversity, coupled with the deeply rooted African ethanobotanic heritage, has already contributed a number of novel chemical entities and still remains a promising untapped reservoir for the discovery of more diverse bioactive molecules. The presentation seeks to reflect the current status and future perspectives of drug discovery in the majority of African countries with emphasis on the present opportunities, strength and weaknesses by providing vivid examples illustrating Africa's efforts to embark into drug discovery from traditional medicines through to natural product-driven search for hits against neglected and non-communicable diseases. Although only a few places in Africa have established the competencies to champion modern drug discovery, the newly emerging initiatives e.g. Drugs for Neglected Diseases *initiative* (DND*i*), currently existing drug discovery networks e.g. Natural Products Research for Eastern and Central Africa (NAPRECA), presently established Africa's first integrated drug discovery and development centre (H3-D), and the most recently launched Pan-African natural product library (P-ANPL) seem to streamline natural products-based drug discovery in Africa in various research platforms involving target identification, hit discovery, lead optimization, modern medicinal chemistry, preclinical pharmacology as well as drug metabolism and pharmacokinetic studies.

L5 [Plenary Lecture] Computational methods in natural products chemistry

Quinn RJ
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The revival of natural products arises because of their novel chemotypes and because of the inherent difficulty associated with producing synthetic libraries that contain molecules that interact with biology space. Natural products have an inherent understanding of biology space. Our lead discovery program is based on the drug-like natural product metabolome. [1, 2] Natural products and their analogues have had high impact as drugs because of the embedded biosynthetic molecular recognition that transfers to therapeutic targets as described by protein fold topology (PFT). [3, 4] The lecture will present our efforts to use computational approaches covering

1. drug-like and lead-like properties
2. natural product scaffold properties using Fsp3 to evaluate structural complexity
3. natural products as lead-structures; chemical transformations to create lead-like libraries.

4. approaches to automate protein fold topology interrogation of large databases.

References: [1] Camp, D.; Davis, R. A.; Campitelli, M.; Ebdon, J.; Quinn, R. J. Drug-like properties: guiding principles for the design of natural product libraries. J. Nat. Prod. 2012, 75, 72 – 81. [2] Camp, D.; Davis, R. A.; Evans-Illidge, E. A.; Quinn, R. J. Guiding principles for natural product drug discovery. Future Med. Chem. 2012, 4, 1067 – 1084. [3] McArdle, B. M.; Campitelli, M. R.; Quinn, R. J. A common Protein Fold Topology shared by flavonoid biosynthetic enzymes and therapeutic targets. J. Nat. Prod. 2006, 69, 14 – 17. [4] Kellenberger, E.; Hofmann, A.; Quinn, R. J. Similar interactions of natural products with biosynthetic enzymes and therapeutic targets could explain why nature produces such a large proportion of existing drugs. Nat. Prod. Rep. 2011, 28, 1483 – 1492.

L6 [Plenary Lecture] Skin active natural products

Rawlings AV
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There are many cosmetic products containing plant-derived extracts that are used to deliver benefits to the consumer. My focus will be centred upon those ingredients that are efficacious for a variety of important consumer problems. Firstly an overview of consumers' needs will be reviewed. Despite the decades of research on cosmetic products consumers still have high expectations of product efficacy. For example, although consumers largely agree that moisturizing technologies are still not meeting their skin desires, differences in the expected performance for antiaging and skin lightening products occur globally. To set the scene a short review of skin structure & function will be given. One or two mechanisms will then be considered from a skin biochemistry perspective and examples will be given for skin active natural products. Nuclear hormone receptors are an important class of transcription factors and one of the most important is the peroxisome proliferator activated receptor class of this transcription factor. The importance of the different isoforms in skin biology will be discussed and agents that influence them. Ligands that active this receptor class will be discussed and examples of their effects in vitro and in vivo will be presented.

L7 [Plenary Lecture] Challenges and pitfalls in pharmacological testing of plant extracts

Butterweck V
University of Applied Sciences Northwestern Switzerland, Muttenz, Switzerland

Pharmacologists have the job to find new therapeutically useful drugs by using appropriate models. These pharmacological models have to be relevant, which means that they should be able to predict clinical efficacy for the intended therapeutic indication. However, one of the major challenges in experimental phytopharmacology is to perform scientifically valid studies with a plant extract since they are multicomponent mixtures of active, partially active, inactive compounds and/or co-effectors. Thus, accurately performed pharmacological experiments rely on critical approaches and well elaborated experimental designs. The experiments not only have to be executed professionally, even more important is the careful and self-critical examination of the results in order to avoid an overinterpretation of the data. Today there is an unfortunate trend to attribute pharmacological activities to almost every plant extract. This leads to serious misinterpretations, especially when conclusions are drawn only based on *in vitro* assays. The challenge for investigators always will be to correlate *in vitro* data with *in vivo* findings. Disease complexity, certainly, represents an obstacle to successful interpretation of data, but methodological pitfalls in development of assays and validation steps also contribute. This lecture will highlight some basic considerations which need to be taken into account when performing pharmacological assays with plant extracts, such as the physicochemical properties of the testing material, choice of realistic doses, adequate test models, appropriate routes of administration, and suitable statistical evaluations for proper data interpretation. Data from the literature as well as findings from our lab will be critically discussed.

Reference: [1] Butterweck and Nahrstedt, Planta Med. 2012, 78(8): 747 – 54.

L8 [Plenary Lecture] The role of non-starchy polysaccharides ("soluble" dietary fibre), in particular (1,3;1,4)-β-glucans and arabinoxylans, in promoting bowel health

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Total dietary fibre contains indigestible plant complex carbohydrates comprising two functionally distinct components, "soluble" and "insoluble" fibre. "Insoluble" fibre (cell walls) provides stool bulk thereby reducing the risk of constipation and diverticulitis. In contrast, high molecular weight (HMW) "soluble" fibre, also referred to as non-starchy polysaccharides (including 1,3;1,4-β-glucans (MLGs) and arabinoxylans (AXs)) lowers the risk of serious diet-related conditions of the developed world, such as Type II diabetes, cardiovascular diseases, colorectal cancer. The grains of cereals are an important source of caloric intake for a major part of the world's population and are also a major source of HMW "soluble" dietary fibre in the form of MLGs and AXs. Recently oats and barley received FDA approval to be labelled as health promoting and we are working towards enhancing the soluble fibre content of wheat, barley and rice endosperm. The overall aim of our work is to determine the molecular mechanism of synthesis and assembly of AXs and MLGs and to use this information to enhance their content in grains through molecular breeding techniques. We are also studying their molecular mechanism(s) in promoting bowel health.

L9 [Highlight Lecture] About the potential of plant senescence as a new source for drug discovery

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Natural products have always been a major source of new lead structures and many of the traditionally applied herbal materials have been well characterised with regard to their phytochemistry. By now, the ongoing search for new drugs and chemical entities has reached exotic and remote organisms that lack traditional application and that are rather hard to supply. However, plant senescence might spotlight herbal materials again as a complementary source for drug discovery. While yet hardly explored, plant senescence appears to involve significant changes of the secondary plant metabolome that may result from the rearrangement of secondary metabolites in the course of nutrient reallocation and from the oxidative conditions in senescing tissue. Besides just quantitatively altering the metabolome, senescence processes in cherry laurel leaves have also proven to result in new natural products that are hardly detectable in green tissue and that allowed new insights on the main constituent's catabolism. The well-established technique of ethylene fumigation is capable of inducing the observed metabolic changes and therefore suggests a straightforward method for the biotechnological production of senescence-associated metabolites. First studies indicate that the phytochemical differences may also impact on the bioactivity of senescent materials. Inclusion of senescent plant material to metabolomic studies aiming at the identification of bioactive constituents may therefore improve the predictive power of supervised multivariate statistical analysis by significantly increasing the variability of both chemical fingerprint and bioactivity data.

AL1

[Award Lecture] Scientifically based, high-quality phytotherapeutics

Apers S
Natural Products and Food – Research and Analysis (NatuRA), University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium

The research carried out in our research group NatuRA, which is a consortium of the Laboratories of Pharmacognosy, Nutrition and Food Science and Pharmaceutical Analysis, is focused on projects in the area of: "Development of scientifically based high-quality phytotherapeutics". More specifically, the consortium has expertise in the following fields: isolation and characterization of secondary metabolites; development and validation of methods for quality control and standardization; investigations of the bioavailability and metabolisation of natural products; and *in vivo* and *in vitro* evaluation of health promoting effects. A major project during the past years was a comprehensive study on saponins isolated from the leaves of *Maesa lanceolata*. After isolating the individual compounds using semi-preparative HPLC, the structures were established using spectroscopic methods. These compounds showed virucidal, haemolytic, molluscicidal and anti-angiogenic activity. Maesasaponin II displayed the highest anti-angiogenic activity, but was only present in very small amounts in the plant. To increase this amount, a combinatorial biosynthesis platform was constructed. For the fast and sensitive analysis of the saponins in the cell cultures a UPLC-MS (TQD) method was developed. A major point of interest within the consortium is phytochemical analysis; i.e. development and validation of methods for quality control and standardization. The laboratory has built up an extensive experience in the analysis of a large variety of natural products; among which, but not limited to phenolic compounds e.g. isoflavones and prenylnaringenins, alkaloids and terpenoidic compounds such as mono-, di and sesquiterpenes and saponins. The mission of the lab is to support, investigate and control the quality of plant based preparations and to contribute to public health. A recent market study of food supplements (Hop, St. John's wort, Ginkgo and Soy) on the Belgian market revealed that only 35% was found to be compliant.

AL2

[Award Lecture] Novel extra-cardiac actions of the *Crataegus* extract WS® 1442: Functional and molecular insights

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Hawthorn (*Crataegus* spp.) extracts belong to the most popular and widely used herbal remedies in the USA and Europe. In a number of countries, the Crataegus special extract WS® 1442 is approved or registered for the treatment of congestive heart failure according to stage II of the New York Heart Association. The efficacy and safety of WS® 1442 for the adjunctive treatment of this disease have been proven by numerous clinical trials and have mainly been ascribed to its positive inotropic, anti-arrhythmic, vasorelaxing, and cardioprotective effects. The extract predominantly contains two groups of polyphenolic compounds, a mixture of monomeric flavonoids and oligomeric procyanidins (OPCs). Interestingly, extra-cardiac effects of the extract are less investigated. In the last years, we disclosed anti-restenotic (1) as well as edema-preventing (2) actions and were able to gain in-depth insights into the underlying molecular mechanisms. Moreover, we were also interested in the involved bioactive compounds. In brief, we could show that (1) orally administered WS® 1442 strongly attenuates neointimal hyperplasia in rat carotid arteries injured by balloon catheter dilatation. Reduction of smooth muscle cell migration and proliferation due to a direct inhibition of PDGFR-? kinase activity were involved in this effect. We also found that (2) the extract effectively protects against endothelial barrier dysfunction by its action on key determinants of endothelial permeability (adherens junctions, actin cytoskeleton, and contractile apparatus). WS® 1442 inhibited the barrier-destabilizing calcium/PKC/Rho signaling by interfering with the endothelial SERCA and IP_3 system and activated the barrier-stabilizing cAMP/Epac1/Rap1 pathway. Upon bio-guided fractionation, OPCs were shown to trigger the cAMP pathway, whereas non-phenolic (yet to be identified) components are responsible for the effects on calcium signaling.

Workshops

Regulatory Affairs Workshop:
Harmonisation of Health Claims in Europe
Chairs: A. Vlietink, S. Alban

8th Young Researchers Workshop
Chairs: D. Tasdemir, A.R. Bilia

Presymposium COST Workshop:
Challenges and Limitations in Metabolic Pathway Engineering of Secondary Natural Products
Chair: O. Kayser

Current Aspects in Manufacturing of Herbal APIs (Extracts):
GACP Regulations/GMP Implementation/Process Development
Chair: C. Erdelmeier

Regulatory Affairs Workshop: Harmonisation of Health Claims in Europe

WS1

Health claims for botanical food supplements: approach and consequences for the regulatory framework

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European Botanical Forum, Brussels, Belgium

In the EU, the use of botanicals for *nutritional or physiological purposes* in food supplements is covered under food law since 2002 (Directive 2002/46). The use of botanicals for *therapeutic or preventive purposes* has been harmonised by the Traditional Herbal Medicinal Product Directive (THMPD, Directive 2004/24). The Court of Justice of the European Union (CJEU) has established the following principles:

- Member Sates must determine the status based on a case-by-case assessment of all of the product's characteristics.
- All products that are presented as having therapeutic or preventative effects should be subject to medicinal law.
- Products having an effect on the human body, but which do not significantly affect the metabolism and thus do not strictly modify the way in which it functions are not medicinal products.

The EU Nutrition and Health Claims Regulation (Regulation 1924/2006) requires scientific studies to prove health benefits, while for botanicals most of the knowledge has been gained through use and experience over time. This was explicitly recognised by the THMP legislation. The requirements for demonstrating simple health effects for food are therefore more demanding than for therapeutic effects for medicinal products. For this reason the European Commission (EC) stopped the process of claims assessment for botanicals in September 2010. In August 2012 the EC published of a discussion document for the Member States, asking for input on one of two options:

- *Option 1:* EFSA to continue the assessment of claims for botanicals as originally foreseen, which would likely reject all health claims for botanicals.
- *Option 2:* Address the specificities of botanicals in specific legislation, taking into consideration traditional use and potentially also safety and quality.

The responses by the Member States diverge and vigorous opposition to option 2 has been expressed by many pharmaceutical stakeholders. Until a decision is taken THMPs and food supplements can continue to be marketed in parallel.

WS2

Efficacy and safety regulations and scientific criteria applicable to traditional botanical food supplements and herbal medicinal products marketed in the European Union

Silano V
II University of Rome, Italy

Highly heterogeneous preparations obtained from many different botanical species and parts and contain-ning many different biologically-active substances have been widely used for long time and are still curren-tly used in EU Member States with the objective of:

- correcting altered physiological processes in case of diseases or preventing their occurence (traditional herbal medicinal products-THMP);

– helping the human body in maintaining its homeostasis, i.e. normal functioning of physiological processes (traditional plant food supplements- TPFS).

Therefore, the use of these preparations has become traditional, in the current European Member countries long before the adoption of the relevant EU regulations mainly depending on the country's availability of botanicals, on prevalent medical and nutritional practices, as well as cultural, technological and social fac-tors peculiar of each country and, therefore, without any coherence among the approaches adopted in differ-rent countries. The European Institutions started their action for harmonizing applicable regulations and scientific criteria to these traditional botanical food supplements and medicinal products only recently (see Directive 2002/46/EC and Directive 2004/24/EC, respectively) and, not surprisingly, with a very limited success. In fact, the distinction and separation of the botanical species and parts being used as traditional food supple-ments and medicinal products in different EU Member States has remained highly problematic, as shown by the fact that a number of botanical species and parts currently used under a specific regulatory domain (e.g. food supplements) in some Member States are used in the other domain (e.g. traditional medi-cinal products) in other Member States, with a very broad overlap. This situation, that has been present almost unnoticed in the European market for long time, is currently challenged by the on going implementation of Regulation (EC) 1924/2006 on nutritional and health claims to botanical food supplements. This presentation intends, first, to provide all the information necessary to characterize the issue and, subsequently, to discuss practicable scientific and regulatory options in the ways forward to deal with this issue that has of significant economic importance as well as some health and safety implications for consumers.

WS3 Why do we have for health claims different criteria between plant based food supplements and herbal medicinal products?

Anton R
University of Strasbourg, France

It seems obvious for most of the people that there are strong differences between botanical food supplements and herbal medicinal products, especially because consumers as well as treatment goals are not the same. However, some main convergences can be observed as for example quality and safety. A key question today is why for a given plant and the same preparation, the requirements imposed by the official European authorities (EMEA) are less severe in terms of clinical assessments to get a marketing authorization for a food product in comparison to a medicinal product? In the EU, traditional herbal medicinal products having a long history of prior human usage can be registered under a special, simplified registration procedure without the necessity of providing some proofs of efficacy with clinical trials. This is the consequence of a specific legal framework known as Directive 2004/24/EC. On the contrary, regarding food products, EFSA requires randomized clinical trials on healthy subjects and under such requirements no "traditional claims" will obtain a positive opinion from this Authority. Furthermore, it is really difficult to understand that for a drug, designed for a targeted disease and focused on a given pharmacological activity, the proof of its efficacy is not required in a contrary to a food supplement, used to maintain the homeostasis status and to help in minor troubles, where the efficacy proof is required. The dosage of a plant extract being lower for a food supplement than for a drug reinforces the difficulty to get the validation. How can we demonstrate such efficacy on a person without evident pathology but having only some well-being problems? In a scientific perspective, this clinical approach is adapted to pure active substances as vitamins but not to the complexity of plant extracts. Many years ago, a group of experts at the Council of Europe proposed some recommendations to differentiate the requirements necessary for food supplements and for herbal drug medicine, taking into consideration for example the individual impact and the final aim of the product. Unfortunately, this proposal has not been retained. In conclusion, we consider today that the requirements necessary to get a validated health claim for botanical food supplements and botanical preparations are not appropriate, leading to an insoluble situation. European harmonization is more and more necessary.

WS4 Herbal medicinal products in the EC; indications versus claims; an up-to-date scientific view

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All over the world medicinal plants have been used in health care since ancient times. Even though selection and documentation of manifold receipts have a long tradition, the formal regulation of herbal medicinal products (HMPs) is newer. The aim of this presentation is mainly to discuss the implementation of the current European regulations at the level of EU authorities in terms of quality, safety and efficacy of herbal products through the HMPs Directive (2004/27EC amending Directive 2001/83/EC as regards Traditional Herbal Medicinal Products (THMPs) and Well Establish Use products (WEU). The Herbal Medicinal Products Committee (HMPC) at the European Medicines Agency (EMA) has adopted guidelines which are intended to support assessment of HMPs considering their particular characteristics. One of the major tasks of the HMPC is to establish community monographs and list entries of herbal substances or combinations. The development of such documents leads to a more harmonised view of the member states and at the end it should ideally generate a common market of HMPs. Currently, about 114 monographs have been finalised and the regulatory framework differentiates between marketing authorisation of HMPs and registration of THMPs, for which the efficacy must be plausible based on their long-standing use instead of being proven by clinical trials. In many cases, the distinction between THMPs and food supplements containing herbal products without nutritional value but having physiological effects, remain difficult and controversial. Borderline products, as well as advantages and disadvantages of both uses (as medicines and nutritional with health claims), have to be pointed out, as well as the consumers protection. A viewpoint of the regulatory Authorities Experience will be discussed in details, through several examples as well as the legal situation in the EU regarding their uses.

8th Young Researchers Workshop

WS5 [Impulse Lecture] Guidelines for a good scientific talk – How to structure and set up a clear research presentation with your own findings

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A major aspect in science is the dissemination of research findings to the scientific community through the participation in conferences, meetings, and workshops. Considering the scientific talk as one of the most important communication media, researchers should be able to deliver a well-organised and clear speech. A good talk given in front of an audience of your research area is an even better self-advertisement than the impact of a scientific paper. However, this is easier said than done. Especially for young scholars at the beginning of their career it might be challenging to develop a certain competence to prepare and deliver a good lecture. It requires the ability to introduce the audience into a specific research area, to give an overview on its current status, to set out the aim of the study, to characterise the methods used, to create a meaningful presentation of the results obtained, and overall, to interpret and critically discuss experiments and outcomes. Moreover, the whole set-up including rhetorical aspects, design of presentation slides, and the non-verbal interaction with the audience play an important role. In this respect, this lecture will provide useful guidelines and suggestions to set up a structured and clear oral presentation – a key qualification for success – by demonstrating the DOs and DON'Ts with some simple examples.

WS6

[Impulse Lecture] Method and result optimizations in the search for cancer chemopreventive natural products

Cuendet M
University of Geneva, University of Lausanne, School of pharmaceutical sciences, Geneva, Switzerland

The treatment of many diseases is highly dependent on natural products, and this is especially true for the treatment of cancer. Most human cancers seem to be potentially preventable because of controllable or removable causative exogenous factors (primary chemoprevention), but also by agents interfering with carcinogenesis. These compounds can be divided into three categories: blocking agents (anti-initiation), anti-promotion agents, and anti-progression agents. Successful hit discovery of cancer chemopreventive candidates relies on rationale strategies involving *in vitro* assays, compound isolation, and *in vivo* studies. Several steps in natural products drug discovery are time consuming, require large amounts of plant material and do not lead to the expected results. Strategies to improve natural products research, such as high-throughput microfractionation coupled to bioassays targeting various stages of carcinogenesis will be shown. Also, various examples of experiments that did not give the expected results and for which careful data analyses and assessment are necessary, will be presented.

WS7

[Impulse Lecture] Perspectives in industry and academia

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Young researchers often have a dream: a scientific career at a high-level international institution, being member of international scientific boards, being a prestigious outstanding scientist, doing excellent science – only for few of them this dream will come true... Young researchers normally have a more concrete idea on the perspectives in academia than in industry due to the fact that they know at least part of the academic environment. Research in academia is focused and financially supported more and more towards interdisciplinary cross-cutting projects in excellence centers. Being part of these project teams or involved in international projects like EU-projects improves a lot of skills besides scientific expertise and is considered as one milestone for scientific career in academia. As is mostly the case young researchers have only vague ideas on the perspectives in industry with regard to the broad spectrum of professional fields which are options for young scientists. Using the example of a mid-sized phyto-pharmaceutical company, attractive departments, functions and positions with their key tasks will be illustrated. Examples for jobs in R&D and in operations, but also in regulatory affairs, medical marketing, in project management and at the interface between departments will be outlined. The implementation of the respective key tasks, what does this mean in practice – "... what's your daily work?" will be touched. Besides formal requirements related to professional expertise, core competencies e.g. communication skills, joined-up thinking, ability to work in a team or solution-oriented approach to work, will be addressed.

WS8

Anti-inflammatory depsides from *Cetrelia monachorum* potently targeting mPGES-1, 5-LO and NF-κB

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Lichens, often underestimated and still underexplored with respect to pharmaceutical lead discovery, provide a vast diversity of small chemical entities with a variety of reported bioactivities. Based on a previously performed pharmacophore-based virtual screening, we identified lichen constituents as potent microsomal prostaglandine E_2 synthase 1 (mPGES-1) inhibitors [1]. Here, we focused on further *in vitro* anti-inflammatory activities of lichen compounds. An *in vitro* screening of 17 Alpine lichen species for inhibition of 5-LO, mPGES-1 and NF-κB revealed *Cetrelia monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. as promising source for novel anti-inflammatory leads. Phytochemical investigation of the ethanolic crude extract resulted in the isolation and identification of 11 constituents, belonging to depsides and derivatives of orsellinic acid, olivetolic acid and olivetol. The two depsides imbricaric acid and perlatolic acid exerted inhibitory activities on mPGES-1 (IC_{50} 1.9 and 0.4 μM, resp) and 5-LO as demonstrated in a cell-based assay (IC_{50} 5.3 and 1.8 μM, resp) and on the purified enzyme (IC_{50} 3.5 and 0.4 μM, resp). Dual inhibition of mPGES-1 and 5-LO provides safer and more effective anti-inflammatory properties [2]. Furthermore, the two main constituents, imbricaric acid and perlatolic acid, quantified in the extract with a content of 15.22% and 9.10%, resp, showed significant inhibition of TNF-α-induced NF-κB activation in luciferase reporter cells with IC_{50} values of 2.0 and 7.0 μM, resp. These findings attest imbricaric acid and perlatolic acid a pronounced threefold anti-inflammatory profile [3], which warrants further investigation on their pharmacokinetics and *in vivo* efficacy. **Acknowledgements:** Supported by the Austrian Science Fund (S10703, S10704). **References:** [1] Bauer J et al. ChemMedChem 2012 7: 2077 – 81. [2] Radmark O, Samuelsson B J Intern Med 2010 268: 5 – 14. [3] Oettl SK et al. 2013 submitted.

WS9

Identification of dihydrostilbenes as a new scaffold for $GABA_A$ receptor modulators in *Pholidota chinensis* stems and roots

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In a search for new natural product-derived $GABA_A$ receptor modulators, we screened a plant extract library on *Xenopus laevis* oocytes expressing recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors, by means of a two-microelectrode voltage clamp assay. A dichloromethane extract of stems and roots of *Pholidota chinensis* (Orchidaceae) enhanced the GABA-induced chloride current (I_{GABA}) by 132.75%± 36.69% at 100 μg/mL. By means of an HPLC-based activity profiling approach, the three structurally related stilbenoids coelonin (**1**), batatasin III (**2**), and pholidotol D (**3**) were identified. Dihydrostilbene **2** enhanced I_{GABA} by 1512.19%± 176.47% at 300 μM, with an EC_{50} of 52.51 ± 16.96 μM, while compounds **1** and **3** showed much lower activity, suggesting conformational flexibility as in **2** to be crucial for receptor modulation. This was confirmed by a study on a series of 11 commercially available stilbenoids and their corresponding semisynthetic dihydro derivatives. When tested at a concentration of 100 μM, dihydrostilbenes showed higher activity in the oocyte assay than their corresponding stilbenes. The dihydro derivatives of tetramethoxy piceatannol (**6**) and pterostilbene (**11**) were the most active, with modulations comparable to that of compound **2** (544.5%± 104.4% and 660.6%± 100.2%, respectively), when tested at the same concentration. Dihydrostilbenes represent a new scaffold for $GABA_A$ receptor modulators.

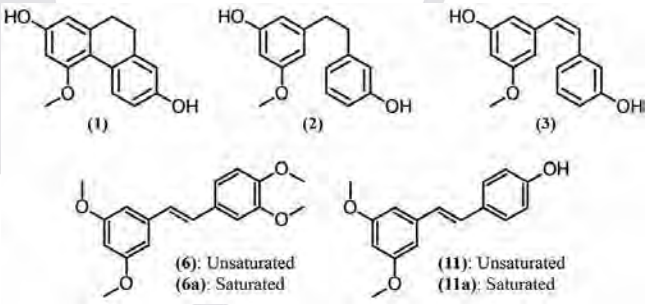

Fig. 1

WS10 Polyphenolic fingerprints of different organs of *Rosa hybrida* cv. 'Jardin de Granville'

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The parfum giant Christian Dior uses *Rosa hybrida* cultivar 'Jardin de Grandville', a delicate clear pink flower, in some luxury cosmetic formulations. This cultivar has been selected not only for its beauty but also for its resistance to diseases. Herein the phytochemical content of 16 different plant organs of this plant [winter woods (ww), shoots (sp sh), early buds (eb), buds before flowering (bbf1), flowers (first flowering period, spring) (flo1), four different flower parts (petals (pet), sepals (sep), receptacles (rec) and stamens (sta)), leaves (lea), summer woods (sw), summer shoots (su sh), buds before flowering (bbf2), flowers (second flowering period, summer) (flo2), roots and fruits] are investigated by RP-UHPLC coupled with DAD and ESI-UHR-Q-TOF-MS. The main focus was the polyphenols that have diverse functions in the plant (e.g. coloration, antioxidant activities, defense molecules against pathogens). By combination of UV, MS data and comparison with reference standards, around 130 phenolic compounds corresponding to tannins derived from quinic, gallic and ellagic acid, quercetin derivatives and kaempferol derivatives were identified. Statistical analyses (PCA, HAC and ANOVA) pointed out clear differences between vegetative (woods, shoots and leaves) and reproductive (flowers and buds) organs. The vegetative part shows a high amount of hydrolysable tannins and catechin derivatives whereas the reproductive organs are richer in specific kaempferol derivatives. Hence some molecules seem to be ubiquitous in the all plant, while other ones could be used as developmental stage markers.

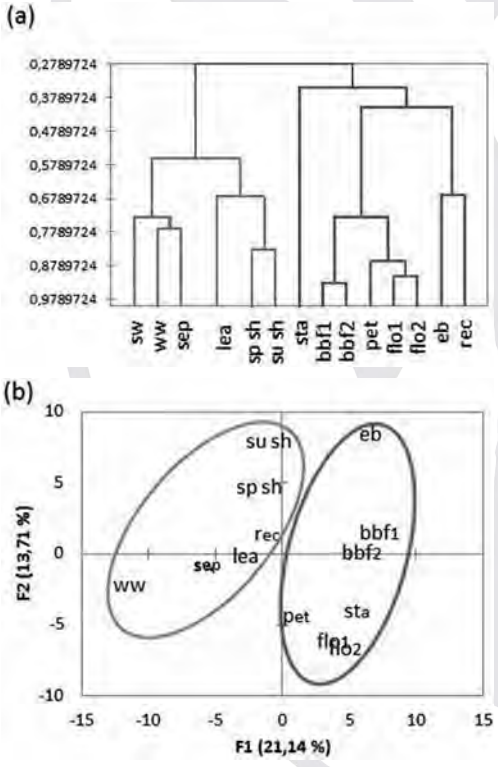

Fig. 1: Statistical analysis of peak areas for 137 secondary metabolites in the different organs of 'Jardin de Granville'.
(a) hierarchical ascendant classification, (b) principal components analysis, score plot.

WS11 Polyphenolic composition and antichlamydial effect of commercial peppermint (*Mentha* x *piperita* L.) teas

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The qualitative and quantitative polyphenolic content of the infusions of the commercial peppermint tea (*Mentha xpiperita* L.) samples (n = 27) from different countries was studied using HPLC-UV-MS/MS analysis. Overall, 22 polyphenols were identified in the peppermint infusions. The major polyphenols were eriocitrin, 12-hydroxyjasmonate sulphate, luteolin-*O*-rutinoside and rosmarinic acid. The total polyphenolic content varied largely among the 27 peppermint tea infusions, found in a range of 10.0 – 218.0 mg/ml. In order to determine the content of samples by finding chemosystematic markers, essential oil composition of the samples was determined by GC. Of the analysed peppermint tea samples, 24 met the standards set by Ph. Eur. 7th Ed., whereas the analyses indicated that three samples may contain *Mentha spicata*, a species different from that claimed on the package. The effects of seven peppermint tea extracts against a respiratory tract pathogen *Chlamydia pneumoniae* were investigated *in vitro*. All the teas prepared from the selected commercial peppermint products inhibited chlamydial growth, inhibitions ranging from 20.7 to 69.5% at the extract concentration of 250 µg/ml. The effect on the inclusion counts at the second passage of infection was studied, showing an inhibitory effect on the infectious progeny production ranging from 7.8 to 78.1%. In most cases, the antichlamydial activity was a characteristic of the peppermint teas having high contents of luteolin and apigenin glycosides. This study supports the consumption of peppermint tea to potentially elicit beneficial health effects on acute respiratory tract infections.

WS12 Occurrence of (R_S,R_C)- and (S_S,R_C)-marasmin in *Tulbaghia violacea* Harv.

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The cysteine sulphoxide marasmin is a natural sulphur-containing aroma precursor, occurring in several plant species, as well as in some members of the genus *Marasmius*. After disruption of a cell containing it, it is cleaved by a C-S-lyase. The generated sulfenic acids lead to the formation of the thiosulfinate marasmicin which is the main odourous compound of *Marasmius* spec. and some of these plants. Marasmicin has been drawn attention to for further investigation due to bioactivity against fungi and *Mycobacterium tuberculosis*. [1] Marasmin was firstly identified as precursor of the garlic odour in *Marasmius* spec. [2]. In the fungi it appears as glutamyl-dipeptide with (S)-configuration at the sulphur atom. 11 years later it was found in the fruits of the tree *Scorodocarpus borneensis* Becc., where it exists in form of the free sulphoxide in (R)-configuration [3]. In the following marasmin was detected in several plants of the *Amaryllidaceae* including *Allium*, *Ipheion*, *Leucocoryne* and *Tulbaghia* [4]. As in *S. borneensis* only the (R_S,R_c)-marasmin could be detected in all plant material investigated so far. Recent research on the South African plant *Tulbaghia violacea* Harv., known as 'society garlic', revealed the presence of both configurations of marasmin (Fig.), with (R_S,R_c)-marasmin being the major compound. This is an example of the rare appearance of both isomers of one cysteine sulphoxide in the same plant as well as the first report of (S_S,R_c)-marasmin within the plant kingdom. This leads to the conclusion that either the oxidizing enzyme is not very specific or there is more than one enzyme involved in the oxidation of cysteine derivatives in this plant.

References: [1] Kusterer J, Fritsch RM, & Keusgen M, *J Agric Food Chem.* (2011) *59*(15):8289 – 97 [2] Gmelin R, Luxa H-H, Roth K, & Höfle G, *Phytochem.* (1976) *15*(11):1717 – 1721 [3] Kubota K, Hirayama H, & Sato Y, *Phytochem.* (1998) *49*(1):99 – 102 [4] Kubec R, Krejčová P, Mansur L, & García N, *J Agric Food Chem.* (2013) *61*(6):1335 – 42

WS13 In vitro antihyperlipidemic activity of triterpenes from stem bark of *Protorhus longifolia* (Benrh) Engl.

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Obesity is a common disorder of carbohydrate and fat metabolism. In an effort to discover new more effective drugs against obesity and its comorbidities, we investigated the *in vitro* antihyperlipidemic activity of two triterpenes (3β-hydroxylanosta-9,24-dien-21-oic acid and methyl-3β-hydroxylanosta-9,24-dienoate isolated from stem bark of *Protorhus longifolia* and characterized through NMR, LC-MS, IR. The inhibitory activity of the triterpenes was evaluated on selected lipid (pancreatic lipase and cholesterol esterase) and carbohydrate (disaccharidases, α-glucosidase) digestive enzymes. The inhibitory activity of the compounds on hormone-sensitive lipase and the ability to bind bile acids were also evaluated. Furthermore, the effect of the compounds on cellular (muscle cells, C2C12 and fat cells, 3T3-L1) glucose uptake was investigated. The triterpenes effectively inhibited the activities of pancreatic lipase, cholesterol esterase, and hormone-sensitive lipase with IC50 values ranging from 26.6 to 430 µg/ml, and showed to varying degree inhibition of maltase and other disaccharidases. The compounds showed moderate bile acid binding ability and at 50 µg/ml, both compounds also mimicked insulin character by effectively stimulating glucose uptake in both the C2C12 and 3T3-L1 cells. It is apparent that the compounds possess hypolipidemic properties.

Challenges and Limitations in Metabolic Pathway Engineering of Secondary Natural Products

WS14 Introduction to COST Action FA1006 – PlantEngine

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A significant amount of knowledge has been gained during the last decades about the biosynthetic capacity of plants and the pathways leading to the formation of plant natural products (PNPs), many of which are of high relevance as pharmaceuticals or fine chemicals for industries. To fully exploit the capacity of engineering plants for the production of high value PNPs, COST Action FA1006, PlantEngine, will support and enhance a Pan-European network which will amalgamate resources, define target pathways and prioritize compounds, disseminate novel technologies and applications, set standards for computational support, and develop synthetic approaches in plant metabolic engineering. The current state of the Action, now in its third year, will be presented.

WS15 Metabolic engineering of volatile production

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Plants are capable of producing and emitting many different volatiles. We focus on Green Leaf Volatiles (GLVs), benzenoids/phenylpropanoids and terpenoids. We are not only interested in their biosynthesis but also in where the synthesis of these volatiles takes place (cells and organelles) and how they and their precursors are transported. Most of this work deals with the biochemistry and molecular biology of plant volatiles that are emitted from vegetative tissues during pathogen- or herbivore-induced stress and the contribution of these volatiles to plant defenses. However, we also focus on floral volatiles in petunia that are mostly involved in attracting pollinators although they are important in deterring florivores too. The topic of my seminar will focus on the regulation of the biosynthesis of these volatiles and the metabolic engineering of volatile production in plants, for example to make them more resistant to herbivores. I will talk about several transcription factors regulating floral volatile benzenoid/phenylpropanoid production in petunia or terpenoid production in glandular trichomes of tomato and how we modified the headspace of petunia and tomato.

WS16 A *Medicago truncatula* ABCG transporter modulates the level of isoflavonoids

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Full size ABCG transporters have been proposed to be implicated in the active transmembrane traffic of various secondary metabolites. Despite the importance of ABCG-based transport for plant-environment interactions, these proteins are still poorly characterised in legumes. The functional characterisation of, recently identified, Medicago ABCG10 was conducted by gene expression analyses, protein localization, as well as metabolites quantification. Biological assays were performed with control and *MtABCG10*-silenced material. The *MtABCG10* mRNA accumulated upon the application of fungal oligosaccharides on plant roots. Spatial expression pattern analysis with a reporter gene revealed that the *MtABCG10* promoter is active mostly within the vascular tissues in various organs. The corresponding protein is located in the plasma membrane. Silencing of *MtABCG10* in hairy roots resulted in lower accumulation of the phenylpropanoid pathway-derived phytoalexin and its precursors. In addition, we observed that *MtABCG10*-silenced composite plants are more susceptible to infection with *Fusarium oxysporum*. Based upon the presented data, we propose that the MtABCG10 is a modulator of isoflavonoid levels during the defence response associated with *de novo* synthesis of *Medicago* phytoalexin. **References:** [1] Banasiak J, Biala W, Staszków A, Swarcewicz B, Kepczynska E, Figlerowicz M, Jasinski M. A Medicago truncatula ABC transporter belonging to subfamily G modulates the level of isoflavonoids. J Exp Bot. 2013 Feb;64(4):1005 – 15 [2] Jasinski M, Banasiak J, Radom M, Kalitkiewicz A, Figlerowicz M. Full-size ABC transporters from the ABCG subfamily in medicago truncatula. Mol Plant Microbe Interact. 2009 Aug;22(8):921 – 31

WS17 Metabolic engineering of terpenoid indole alkaloid pathway in *Catharanthus roseus*

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Eukaryotes such as higher plants have evolved to produce a diverse range of low-molecular-weight secondary compounds that can be used as food and feed additives, flavours, fragrances, cosmetics, agrochemicals and pharmaceuticals. The dominant role of secondary metabolites in the pharmaceutical industry is demonstrated by the fact that approximately 50% of novel anticancer drugs have been discovered from nature including blockbusters such as taxanes (paclitaxel), *Catharanthus* alkaloids and camptothecin. The chemical synthesis of plant-derived compounds is usually challenging and uneconomical because the complex stereospecific structures are difficult to replicate. Sustainable and cost-effective production systems must therefore be developed, and the best outcome can be achieved by integrating biotechnology-based approaches into more sustainable production chains featuring cutting-edge innovative technologies. Spectacular advances in characterizing plant metabolic pathways using functional genomics and through the development of large-scale cultivation processes have offered for the first time unprecedented opportunities to explore the extraordinary complexity of the biochemical capacity of plants in entirely new ways. State-of-the-art genomics tools can now be used to improve the production of known natural compounds or to synthesize entirely novel plant constituents by combinatorial biochemistry in cultivated plants and cells[1]. Therefore, the utilization of plants and cells as green production factories is becoming more realistic and more attractive also from a commercial point of view. Metabolic engineering aspects to discover bottlenecks in the biosynthetic pathways and direct the selected pathway towards the desired end-product will be discussed using examples of our large EU-project consortium SmartCell which focuses on terpenoid indole alkaloids. **Reference:** [1] Rischer H, Häkkinen ST, Ritala A, Seppänen-Laakso T, Miralpeix B, Capell T, Christou P, Oksman-Caldentey K-M (2013): Plant cells as pharmaceutical factories. Curr Pharm Design (in press).

WS18 GondenBraid2.0: A comprehensive toolkit for plant synthetic biology

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Plant Synthetic Biology (PSB) aims to design plants with novel traits by introducing new biological parts or systems or by redesigning existing ones to carry out novel tasks. This will be fostered by the employ of modular DNA assembly systems that facilitate the writing of complex genetic instructions while enabling the exchange and reuse of new synthetic modules. GoldenBraid (GB) [1] is a modular DNA assembly method created to overcome the existing limitations for multigene engineering in plants. GB iterative cloning loop is based on a very efficient digestion/ligation method [2,3] that turns into a routine the assembly of combinatorial constructs. The GB cloning kit comprises a set of eight destination plasmids (pDGBs) designed to host scar-benign multipartite composites that can be binarily combined to create complex multigene constructs. GB makes possible the assembly of 15 – 19 kb constructs comprising 4 – 5 transcription units made of individual standardized GBparts in a few days work. A recently released new version of GoldenBraid, named GB2.0, [4] proposes a modular cloning schema with positional notation that resembles the grammar of natural languages. Use of the GB2.0 framework is facilitated by a number of web resources (www.gbcloning.org), which include a publicly available database, tutorials and a software package that provides *in silico* simulations and lab protocols. A growing number of vector backbones, constitutive and inducible promoters, reporters, tags, marker genes and silencing tools (amiRNA, tasiRNA, hpRNA) among other elements complete the GB2.0 framework, which aims to serve as a reference for Plant Synthetic Biologists. **References:** [1] Sarrion-Perdigones (2011). PLoSOne6: e21622. [2] Engler (2008). PLoSOne3: e3647. [3] Engler (2009). PLoSOne4: e5553. [4] Sarrión-Perdigones (2012). Plant Physiol. In press.

WS19 Synergy and antagonism of active constituents in a complex herbal formulation on metabolic regulation at a transcriptional level

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Gene expression profiling was conducted on the human neuroglial cell line, T98G, after treatment with either a complex herbal formulation (ADAPT-232) or its constituents, which included extracts of Eleutherococcus senticosus root, Schisandra chinensis berry, and Rhodiola rosea root or several individual constituents, including eleutheroside E, schizandrin B, salidroside, triandrin, and tyrosol. The concentration at which the compounds were tested strongly influenced both the intensity of the cellular response and the profile of differentially expressed genes. Combining two or more active substances in one mixture significantly changed deregulated gene profiles: synergetic interactions resulted in activation of genes that none of the individual substances affected; antagonistic interactions resulted in suppression of some genes that had been activated by the individual substances. These interactions may influence transcriptional control of metabolic regulation, on both the cellular and the whole organism levels. This study was the first to demonstrate that combining active substances with different deregulated gene array profiles and intracellular networks could produce a new substance with unique pharmacological characteristics. Thus, the mixture of two chemical substances could produce a qualitatively new substance, biologically different from its constituents. Presumably, this phenomenon could be used to eliminate undesirable effects (e.g. toxic effects) and increase the selectivity of pharmacological interventions

WS20 *Mucuna pruriens* (Kewanch), the L-DOPA (anti parkinson's drug) producing plant species: current scenario and future prospects

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Mucuna pruriens, the climbing vines and cover crop, found worldwide in tropical areas, is known to produce L-DOPA (L-3, 4-dihydroxy phenylalanine), a potent neurotransmitter, used for the treatment of Parkinson's disease. All parts of *M. pruriens* possess valuable medicinal properties like analgesic, anti-inflammatory, anti-diabetic, neuroprotective, learning, memory enhancement, antihelmintic activity, aphrodisiac, anti-neoplastic, anti-epileptic and antimicrobial activities. These are showing the multifaceted use and globally increased demand in pharmaceutical industry, which is largely met through wild populations. The trichomes present on the pods can cause severe itching, blisters and dermatitis, discouraging cultivation. Therefore, a core collection of mutants were developed and screened for elite accessions with desired high yielding traits from trichome less variety of CSIR-CIMAP (CIM-AJAR). The selected elite accessions will be analysed for morphological characters, molecular polymorphism and subjected to field trials for testing the yield potential and various new unexplored bioactivities. The therapeutic effecthas been attributed to a combination of unidentified substances and L-DOPA, found to be more effective than compatible doses of levodopa in Parkinson's disease and does not induce dyskinesia. It should be validated according to Ayurveda and modern biotechnological tools. By utilizing the genomics interventions, genetically improved elite genotypes could be developed and newer information will be generated by analyzing differential transcripts. This integrated approach provides a mechanism to understand and enhance biosynthesis of L DOPA *in planta*.

Biotechnological explorations	Current scenario	Future requirement
Genetic improvement	Progress	Enhanced yield
Genetic diversity	Compared	Analysis required
Biosynthetic pathway	Less information	-
Expression analysis	Progress	-
Novel sequences	Less	-
Bioactivities	Progress	-

Fig. 1

Current Aspects in Manufacturing of Herbal APIs (Extracts): GACP Regulations/GMP Implementation/Process Development

WS22 GACP regulation for herbal raw materials

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The composition of herbal raw materials is the key driver for the quality of everything manufactured from them [1]. This is why the robust supply of herbal raw materials for the production of both, extracts for phyto-pharmaceutical products as well as for natural compounds, which are subsequently used either directly as APIs or even for derivatives, requires rules of the game. Those have been set up by EUROPAM [2] in 1998 and adapted to the EMA guideline on good agricultural and collection Practice (GACP) for starting materials of herbal origin [3] in 2006. Their compilation led to a price increase due to implementation of QM systems on the farms and additional work because of a rise in documen-

tation. Nevertheless they are an essential part of the overall production process as they intrinsic lead to reliable quality. A meaningful and appropriate elaborated QM system helps economic improvement of processes and quality [4]. Hence, the obligation to work according to the guideline boosts the chance to produce the crop more tailor-made and enables companies to compete in the market with unique products. **References:** [1] Brand, N.; Brückner, T.; Gaedcke, F.; Steinhoff, B. Pharm Ind 71 (2009) 490 – 500 [2] Guidelines for Good Agricultural and Wild Collection Practices for Medicinal and Aromatic Plants (GACP-MAP) EUROPAM, 8th ed. (2010) http://www.europam.net/documents/gacp/EUROPAM_GACP_MAP_8.0.pdf [3] HMPC Guideline on Good Agricultural and Collection Practice (GACP) for Starting Materials Of Herbal Origin; EMA (2006) http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003362.pdf [4] Graf vom Hagen-Plettenberg M, Klier B, Tegtmeier M, Waimer F, Steinhoff B. Z ARZNEIGEWURZPFLA (2012);17:105 – 108.

WS23 Implementation of GMP in manufacturing of herbal APIs (extracts)

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Herbal medicinal products comprise a multitude of herbal preparations as active substances. All these preparations require a specific manner of supply, production, equipment, quality control, storage, etc. Good Agricultural and Collection Practice (GACP) deals with the early steps of the supply chain and is specifically tailored to reflect the particularities of the herbal starting material. The EU-GMP Guidelines and the Annex 7 to these Guidelines regulate the steps following the primary processing covered by GACP. Unfortunately, the GMP Guideline itself gives no clear distinction between chemically defined APIs and herbal preparations serving as APIs, but it exempts the early process steps, e.g. cutting, windsifting, extraction from the scope of GMP. Contrary to this, the Annex 7 to the EU-GMP Guideline intends to include these early steps into the scope of GMP. As a result, there is an on-going discussion about the applicability of the Guideline especially for the early process steps in the manufacturing of herbal preparations. Even if Annex 7 assigns those steps to GMP, it is clear that the requirements of the Guideline cannot be transferred "as such", but need to be adapted in order to reflect the nature of the material and the specific processes. Thus, according to his knowledge the manufacturer assigns the starting point and the extent of GMP applicability on a case-by-case basis depending on the particularities of the product and the process. As well, he ensures that the extent of GMP increases along the process chain reaching full GMP level at least for the final steps, e.g. milling, blending and packaging of the API. Best practice approaches and examples for cleaning validation, qualification of equipment, in process control etc. show that there is specific expertise required in order to establish an appropriate quality management system along the production and quality control of herbal APIs reflecting the specifities of the starting materials as well as the processes applied.

WS24 QBD in process design for phytoextracts

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In the current time of increasing costs for mineral resources, plants come back into focus of even the chemical industry as their use can assure sustainability and environmental compatibility. Plant constituents are used to substitute basic chemicals of petrochemical origin more and more. The first step for preparing the desired natural constituents is always an extraction. [1] Today the central aim for research and development of extraction procedures are safe, efficient and successful processes. Their use guarantees the necessary high quality and attractive economic efficiency in the use of plant extracts. In recent years, process design as well as optimization of existing processes is supported by theoretical modeling the unit operations. Therefore, model parameters should be determined in lab scale [2]. On the other hand, new principles in the research are generated, which allow a rapid screening of possible conditions for extraction in the view of basic processes, solvents, temperatures and pressures. So also the complex character of plant extracts is considered, which is determined by the multicomponent mixture of groups constituents of interest and also the side-fractions. The extraction process has to guarantee that side-fractions are not critical in the subsequent use of the plant extract. Selective extractions should therefore assure that problematic fractions are not co-extracted [3]. **References:** [1] http://www.processnet.org/dechema_media/Downloads/Positionspapiere/PP+ Phytoextrakte +Okt+2012.pdf [2] Josch, J.P. et al, *Food and Nutricion Science*, 3 (6): **2012** [3] Tegtmeier, M., *Chemie Ingenieur Technik* 84 (6): 1 – 4. **2012**

WS25 Regulatory viewpoint

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Short lectures

Topics:
- Natural products against neglected diseases
- In vivo phytopharmacology
- Skin-active natural products
- Ethnopharmacology of African medicine
- Ethnopharmacology of Amazonian medicine
- Computational methods in natural products chemistry
- Hyphenated analytical techniques and target fishing
- Natural product chemistry
- Plant phenols: structures, analytics and activities
- Quality control methods for medicinal plants, extracts and isolated natural products
- Herbal medicinal products in animal healthcare and veterinary medicine
- Miscellaneous

Natural products against neglected diseases

SL1 Oxidative stress induced in *Leishmania infatum* by batzelladine L and norbatzelladine L alkaloids isolated from the marine sponge *Monanchora arbuscula[manuell*

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Leishmaniasis and American Trypanosomiais are protozoan neglected diseases affecting about 12 and 28 million people yearly in developing countries, respectively, with few and toxic alternatives for treatment. The study of new cell death pathways in protozoan parasites is an important strategy to control the progress of infectious diseases. In the search towards new drug leads to treat parasitic infectious diseases, marine natural products are considered promising tools for the discovery of drug prototypes. In the present investigation, the alkaloids batzelladine L and norbatzelladine L, isolated from the marine sponge *Monanchora arbuscula*, were tested against *Leishmania infantum* and *T. cruzi* parasites. Both alkaloids displayed IC_{50} in the range between 1 to 4 µg/mL, with an IC_{50} against a mammalian cell in the range between of 13 to 44 µg/mL. Batzelladine L and norbatzelladine L altered the permeability of *Leishmania* plasma membrane, but norbatzelladine L induced a time-dependent and the highest penetration of the fluorescent dye SYTOX Green. Both batzelladine L and norbatzelladine L induced rapid depolarization of *Leishmania* mitochondrial membrane potential, resulting in an up-regulation of reactive oxygen species (ROS), leading to oxidative stress. Moreover, batzelladine L demonstrated the highest capacity to induce ROS production, in a 4,300-fold higher levels when compared to untreated parasites. Flow cytometry analysis demonstrated an intense exposure of phospatidylserine in the outer leaflet of plasma membrane of batzelladine L-treated parasites. The size of the side alkyl chain of the

two guanidine alkaloids batzelladine L and norbatzelladine L seems to play a major role in the antiparasitic effect. Both alkaloids represent promising tools to study novel cellular death pathways in *Leishmania* parasites.

SL2 Antiprotozoal isoflavan quinones from *Abrus precatorius*

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A library of 309 extracts from selected South African plants was screened *in vitro* against a panel of protozoan parasites. A CH_2Cl_2/MeOH (1:1) extract of the whole plant of *Abrus precatorius* L. *ssp. africanus* Verdc. (Fabaceae) inhibited *Plasmodium falciparum* (97.8%), *Trypanosoma brucei rhodesiense* (100%), and *Leishmania donovani* (75.5%) when tested at 4.8 mg/mL. Active constituents in two different batches of plant material were tracked by HPLC-based activity profiling, and isolated by normal phase flash chromatography and RP-HPLC. Structures and relative configuration of compounds were established by NMR (^{1}H, ^{13}C, COSY, HMBC, HSQC, NOE difference). The absolute configuration was determined by comparison of electronic circular dichroism (ECD) spectra with calculated ECD data. Ten compounds (Fig. 1) were obtained and identified as isoflavan quinones and hydroquinones, among them five new natural products. Abruquinone I (**1**) and abruquinone B (**8**) showed strong *in vitro* activity against *T. brucei rhodesiense* (IC_{50}s of 0.30 µM ± 0.1 and 0.16 µM ± 0.1, respectively). Selectivity indices (SI) as calculated from cytotoxicity data in L-6 cells were 78.3 and 61.3. These SI qualify **1** and **8** as good candidates for assessment of *in vivo* activity. In contrast, compound **2** was not selective, even though very active (IC_{50} 0.88 µM ± 0.1; SI 4.5) [1]. Given that **1** and **8** possess a tenfold higher SI than cynaropicrin, the only plant derived compound with demonstrated *in vivo* activity against *T. b. rhodesiense* [2], **1** and **8** are of considerable interest.

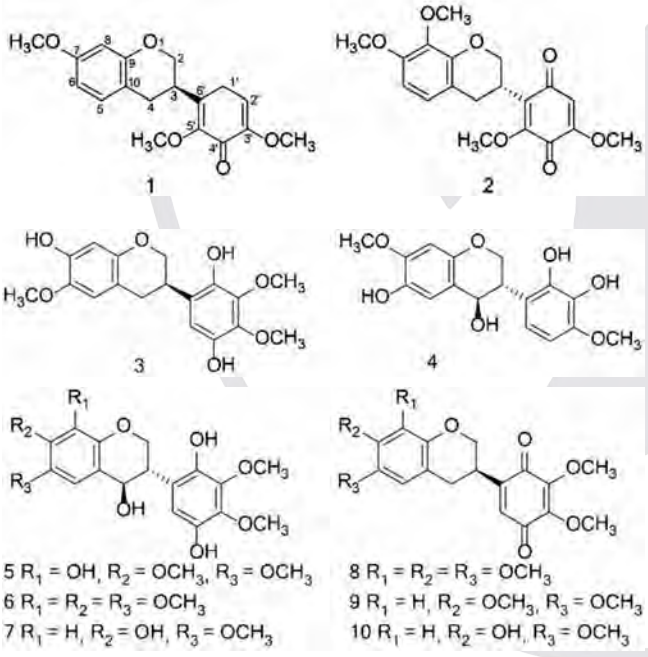

Fig. 1

References: [1] Hata Y, Raith M, Ebrahimi SN, Zimmermann S, Mokoka T, Naidoo D, Fouche G, Maharaj V, Kaiser M, Brun R, Hamburger M. Antiprotozoal isoflavan quinones from *Abrus precatorius* ssp. *africanus*. Planta Med, 2013, DOI: 10.1055/s-0032 – 1328298. [2] Zimmermann S, Kaiser M, Brun R, Hamburger M, Adams M. Cynaropicrin: the first natural product with in vivo activity against *Trypanosoma brucei rhodesiense*. Planta Med, 2012; 78: 553 – 556.

SL3 Trypanocidal activity of flavonoids from *Vitex simplicifolia*

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Trypanosomiasis is one of the public health problems, especially in tropical and subtropical regions and has been categorised by WHO as one of the neglected diseases. Therefore the search for novel, effective and safer drugs for the treatment of this disease continues [1]. A number of studies revealed that many plants are potential sources of novel trypanocidal compounds[2]. We carried out *in vitro* and *in vivo* studies to determine the antitrypanosomal effects of the methanol extract of *Vitex simplicifolia* using *Trypanosoma brucei brucei* infected mice and *Trypanosoma brucei rhodesiense*. Successive extractions with hexane, dichloromethane (DCM), ethyl acetate, n-butanol and water coupled with activity guided fractionation proved the bioactivity of the DCM fraction. Further analysis of the DCM fraction by vacuum liquid chromatography, gel filtration using sephadex LH20, monitored with HPLC was followed by semi preparative HPLC to yield six pure compounds including 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4 H-chromen-4-one **(1),** 3,5,7,8-tetrahydroxy-2-(4-methoxyphenyl) chroman-4-one **(2),** 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-4 H-chromen-4-one **(3),** 5-hydroxy-2-(4-hydroxyphenyl)-3,6,7-trimethoxy-4 H-chromen-4-one **(4),** 5-hydroxy– 2-(4-hydroxyphenyl)-3,7-dimethyoxy-4 H-chromen-4-one **(5)** and 2-(3,4-dimethoxyphenyl)-7-hydroxy-4 H-chromen-4-one **(6)**. The structures of the isolated compounds were elucidated by 1- and 2D NMR spectroscopy, mass spectrometry as well as comparison with literature data. Compounds **4 – 6** exhibited significant trypanocidal activities ranging from 6.43 to 12.3 µg/ml and the cytotoxicity ranges from 1.58 to 6.64 µg/ml. This is the first report of trypanocidal effect of flavonoids from this plant. **References:** [1] Nwodo *et al. Afr. J. Pharm. Res. Dev.* 2012, 4(1) 35 – 40 [2] Hoet *et al., Nat. Prod. Rep*. 2004, 21, 353 – 364.

SL4 Screening of some Asteraceae to discover new active compounds against *Trypanosoma brucei* by metabolite profiling and PLS analysis

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Trypanosoma brucei (*T.b.*) is the etiologic agent of Human African Trypanosomiasis (HAT, "sleeping sickness"). Available drugs for treatment of HAT being limited and toxic, there is a need to search new active compounds which might lead to safer and more effective drugs. Natural products are a rich source of new antiprotozoal leads [1], and Asteraceae plants have repeatedly shown antiprotozoal activity in our previously studies [2, 3]. In this work extracts of flowers and leaves of 7 *Viguiera* species occurring in Brazil were prepared with solvents of different polarity. They were assayed in vitro against *T.b. rhodesiense* and analyzed by UPLC/ESI-qTOF-MS. Biological activity data (% growth inhibition (GI) at 10 µg/mL) of 38 extracts were correlated with the LC/MS data by partial least squares regression (PLS). A good separation of active (> 60% GI) and non-active samples (R = 0.91, Q = 0.71) was obtained with three PLS components. Comparison of the scores- and loadings plots allowed the localization of compounds that are likely to be responsible for high activity of different samples. The constituents highlighted by the PLS analysis comprise several kaurene and pimarane diterpenes as

well as some unknown constituents. In case of the extracts of *V. linearifolia*, the compound responsible for the high anti-*T.b.* activity was identified as the sesquiterpene lactone budlein A (**1**), which was recently demonstrated to be an exceptionally strong anti-*T.b.*-agent [1, 4]. This result confirms the usefulness of the approach used here, which is less time-consuming than classical methods and provides the possibility for a targeted isolation avoiding compounds of low interest.

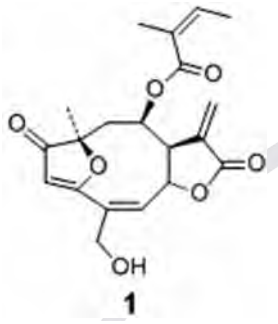

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Fig. 1

References: [1] Schmidt TJ et al. 2012, Curr Med Chem, 19, 2128 – 75 and 2176 – 228 [2] Nour AMM et al. 2009, Planta Med 75, 1363 – 78 [3] Gökbulut et al., 2012, Planta Med 78, 225 – 9 [4] Schmidt TJ et al. Abstract, GA meeting 2013 This work is part of the activities of ResNetNPND: http://www.uni-muenster.de/ResNetNPND

SL5 Oxidative stress induced by (-)-elatol leads to autophagic death in amastigote forms of *Trypanosoma cruzi*

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Chagas' disease, a neglected disease, is caused by the parasite *Trypanosoma cruzi*. The currently drugs available for the treatment of this disease are unsatisfactory, making the search for new chemotherapeutic agents a priority [1, 2]. Recently we described the trypanocidal action of (-)-elatol, extracted from macroalgae *Laurencia dendroidea* [3]. However, nothing was described about the mechanism of action of this compound on amastigotes that are involved in the chronic phase of Chagas' disease. Thus, this study evaluated the effect of (-)-elatol on formation of superoxide anion ($O_2^{\cdot-}$), DNA fragmentation and autophagy in amastigotes of *T. cruzi*. Amastigotes were loaded with MitoSOX and then washed with Krebs-Henseleit buffer before the assays. The formation of $O_2^{\cdot-}$ was evaluated during the exposure of amastigotes to 3, 15, 30 and 150 µM of (-)-elatol and after different times (0, 1, 2 and 3 h) of incubation the fluorescence was measured by fluorimetry. Amastigotes were also treated with 1.5 and 3 µM of (-)-elatol for 24 h and labeled with TUNEL to measure DNA fragmentation or incubated with monodansylcadaverine to determine autophagic vacuoles using fluorescence microscope. The treatment of amastigotes with (-)-elatol induced increase in the formation of $O_2^{\cdot-}$ in all concentrations of (-)-elatol assayed, unlike the untreated parasites. Additionally, an increase of fluorescence was observed in treated parasites with (-)-elatol indicating DNA fragmentation and formation of autophagic vacuoles. It is possible to suppose that the trypanocidal action of (-)-elatol might induce autophagic death pathway triggered by an imbalance of the parasite redox metabolism. **References:** [1] E. Izumi, et al., *Experimental Parasitology*, 118 (2008) 324 – 330. [2] P., Veiga-Santos et al., *Parasitology*, 137 (2010) 1661 – 1670. [3] V. C., Desoti et al., *Marine Drugs*, 10 (2012) 1631 – 1646, 2012. **Acknowledgements:** *This study was supported through grants from CNPq, Fundação Araucária, FINEP, and CAPES.*

SL6 Potent antiplasmodial agents in *Carica papaya* L.

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Decoctions of papaya leaves have been used in Indonesia as a traditional remedy to prevent and treat malaria [1]. With the aim to validate the traditional use we tested a methanolic extract of *Carica papaya* L. against *Plasmodium falciparum* (K1 strain) *in vitro*. This extract inhibited the growth of the parasites by 51% at a test concentration of 4.8 µg/ml. HPLC-based activity profiling located the active compounds in the extract as alkaloids. After enrichment of these alkaloids with cationic ion exchange resin and separation of the alkaloidal fraction with ELSD-triggered flash chromatography, we successfully isolated five piperidine alkaloids. By means of spectroscopic and computational methods, corroborated by X-ray analysis, the structures of (-)-carpamic acid, (+)-methyl carpamate, (+)-carpaine [2], along with a (+)-stereoisomer of carpaine and a (+)-derivative of carpaine produced by monomethanolysis were identified. When tested against *Plasmodium falciparum* (K1 strain), (+)-carpaine (IC_{50} of 0.21 µM, selectivity index of 98) showed the most potent and selective antiplasmodial *in vitro* activity amongst the isolated compounds. Despite this very promising result, *in vivo* testing of carpaine in a murine model (daily dose of 10 mg/kg BW intraperitoneally) did not reduce parasitemia until day 10 after infection. **References:** [1] Rehena, JF. The effect of papaya leaf extracts (*Carica papaya* L.) to the growth of malarial parasite and its socialization as antimalarial for the society in Kairatu sub-disctrict, West Seram district. [Master Thesis] Malang State University, Malang; 2009 [2] Coke, JL., Rice, WY. Jr.. The absolute configuration of carpaine. J Org Chem 1965; 30: 3420 – 2

In vivo phytopharmacology

SL7 A multitarget approach by STW5: the inhibition of a proinflammatory signature, the increase of membrane-bound mucins and the restoration of barrier function improves esophagitis in rats

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Gastroesophageal reflux disease (GERD) is one of the most common GI-diagnosis. Proton pump inhibitors (PPIs) constitute the main treatment, but up to 40% of patients do not achieve adequate symptom control. STW5, a multi-component herbal preparation, was shown to relief concomitant reflux symptoms in patients with functional dyspepsia. Therefore, the efficacy of STW5 was assessed in a subchronic model of GERD. Rats were pre-treated for 7 d with STW5 (0.5 or 2 ml/kg) or Omeprazole (O). Esophagitis was induced surgically and treatment continued for further 10 d before rats were sacrificed and all esophagi were excised. RNA was isolated from defined tissue areas and analyzed by Agilent whole genome microarray. Vehicle treated controls showed marked esophagitis accompanied by significant upregulation of transcripts of well-known markers of inflammation like IL-1ß (14x), IL-6 (11x), TNF-α (11x), CINC-1,2&3 (IL-8 analoga) and transcripts so far not linked to esophagitis like CXCL1 (CXC-motif ligand 1, melanoma growth factor; 32x) or CCL4 (macrophage inflammatory protein 1; 34x). Both treatments improved esophagitis. Proteom-profiling of tissue homogenates revealed a stronger and dose-dependent downregulation of proinflammatory mediators like IL-1ß or CXCL1 in the STW5 groups compared to O. This was also reflected in the transcript profile. IL-1ß and especially members of the C- and CXC chemokine families (CCL4, CXCL1, 2, 3, 6 & 10) were strongly downregulated (10 to 33x). IL-6 and TNF-a were down regu-

lated by STW5 in the range of their upregulation. In addition, the transmembrane mucin MUC 16 and members of the claudin family (Claudin 3, 23), crucial in the formation and maintenance of epithelial barriers were modulated in the range of their dysregulation. Results further substantiate the clinical data on STW5 and might pave the way for clinical use of multi-target agents like STW5 in the treatment of GERD, especially in patients not adequately controlled by PPIs.

SL8 Comfrey root extract in combination with methylnicotinate in the treatment of acute upper or lower back pain: Results of a double-blind, placebo-controlled, multicenter, three-arm RCT

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Several RCTs have confirmed the efficacy of comfrey root extract *(Symphytum officinale* L.) (CRE) in painful muscle and joint complaints. The topical pharmacotherapeutic approach has also included hyperaemising drugs to relax the contracted muscle area. Thus, the extract has also been used in combination with methylnicotinate (MN). The objective was to show the superiority of a CRE plus MN cream (Kytta-Balsam® f) to MN and placebo cream in patients with acute upper or low back pain. In this RCT 379 patients were assigned to three groups (combination of 35% CRE plus 1.2% MN, n = 163; MN, n = 164; placebo, n = 52) and applied a 12 cm layer of cream 3 times daily for 5 days. The trial included four visits. The primary efficacy variable was the AUC (area under the curve) of the VAS (Visual Analogue Scale) on active standardised movement values at visits 1 to 4. Patients performed standardised, muscle group specific tests. Secondary measures included back pain at rest, pressure algometry, consumption of analgesic medication, functional impairment measured with Oswestry Disability Index, and global assessment of response. The AUC of the VAS on active standardised movement was markedly smaller in the combination treatment group than in the MN and the placebo group (ANOVA: $p < 0.0001$). The pairwise comparisons of the mean AUCs of VAS sums showed values 27% lower in favour of the combination compared to MN, and values 50% lower in favour of the combination compared to placebo. MN alone reached a reduction in this variable of 31% compared to placebo. All pairwise comparisons were statistically significant (t-test: $p < 0.0001$). The results were consistent across the primary and all secondary variables. Patients treated with the combination had significant superior reductions in pain scores and were more satisfied with the treatment effect. The combination demonstrated superiority to the two other treatment arms, while methyl nicotinate displayed a considerable effect as well.

SL9 Memory and cognition enhancing activities of an ethanolic extract from *Calendula officinalis* flowers after oral administration in rats and mice

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Pharmaceutical preparations containing *Calendula officinalis* have a long tradition in the treatment of wounds and other skin irritations. Beside topical application, internal administration is also employed to treat gastro-intestinal disorders, amenorrhoea or epistaxis. During a systematic evaluation of oral uses of *C. officinalis* in folk medicine, we gained information suggesting that such extracts cause sedation. Based on these observations, we investigated if an ethanolic (60% m/m) extract prepared from the flowers of C. officinalis (CoE) has pharmacological effects on the CNS. Surprisingly, we obtained data indicating clinically relevant improvement of cognitive functions. Behavior of young and old mice was tested in the T-maze which is a suitable animal model to study spatial working memory. Oral administration of CoE at doses of 150 and 300 mg/kg antagonized scopolamine-induced or age-dependent cognitive impairment comparable to the 0.3 mg/kg of the positive reference drug donepezil (73%, 90%, 77%) respectively. In addition, rats were tested in the Morris water maze and the passive avoidance test. Both models are often used to investigate learning behavior of rodents. Cognitive impairment was induced by the mitochondrial poison sodium azide (NaN_3) which was administered via subcutaneously implanted osmotic minipumps. Daily administration of CoE at doses of 50, 100 and 200 mg/kg/day for 30 days prevented NaN_3-induced deficits in the Morris maze in a dose-dependent manner by 68%, 85% and 88%, respectively. In the passive avoidance model all three doses completely reversed the NaN_3-induced impairments. Initial studies on the mechanism of action showed that administration of CoE modulates the plasma ratio of dehydroepiandrosterone (DHEA) to DHEA sulfate which was accompanied by a moderately increased hippocampal acetylcholine concentration. These results indicate that CoE enhances brain cholinergic function and improves memory, learning and attention in rodents.

SL10 Antidiabetic activity and chemical characterization of the *Acalypha wilkesiana* (Euphorbiaceae) Mull Arg. roots in alloxan-induced diabetic rats

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The aim of this study was to investigate the anti-diabetic effects of methanol root extract of *Acalypha wilkesiana* in normal and alloxan-induced diabetic rats. The effect of the extract (100 and 200 mg/kg body weight, i.p) on fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT0 level, and liver glycogen content were investigated in alloxan induced diabetic rats and found significant effects. A comparison was made between the action of the extract and a known anti-diabetic drug glibenclamide (5 mg/kg b. wt.). An oral glucose tolerance test (OGTT) was also performed in diabetic rats. The standardization of the root was done by following pharmacognostical screening methods. Dose selection was made on the basis of acute oral toxicity study. The most significant reduction of FBG level of 48.36% was observed for 200 mg/g in alloxan induced diabetic rats. A significant reduction ($p < 0.05$) in serum TC and TG level of 50.43% and 58.05% respectively was also observed for the high dose. In diabetic rats, SGOT and SGPT levels were significantly reduced. The methanol extract also showed improvement in parameters like body weight as well as regeneration of β-cells of pancreas in diabetic rats. A histopathological study shows the healing of pancreas by the extract, as a possible mechanism of their antidiabetic activity. These results indicate that the methanol root extract of *Acalypha wilkesiana,* have favorable effects in bringing down the severity of diabetes together with hepatoprotectivity and justify its use in traditional medicine for the treatment of diabetes.

SL11 Comparative effects of STW 5 and STW 5-II in dextran sodium sulfate-induced colitis

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STW 5 (Iberogast®) is an herbal preparation consisting of nine different extracts namely, *Iberis amara*, *Matricaria recutita*, *Carum carvi*, *Melissa officinalis*, *Mentha piperita*, *Glycyrrhiza glabra, Silybum marianum*, *Angelica archangelica* and *Chelidonium majus*. A sister preparation, STW 5-II, has been developed lacking the 3 last components. Both STW 5 and STW 5-II were found effective clinically in gastrointestinal disorders such as functional dyspepsia and irritable bowel syndrome (1,2,3). We have recently shown that STW 5 is useful in experimentally induced ulcerative colitis (4). Present study compares the effect of both preparations in colitis induced in Wistar rats by giving them 5% Dextran sodium sulfate (DSS) in drinking water for 1 week. Treated rats were given either STW 5 or STW 5-II (2 ml/Kg) orally during DSS administration and for 1 week after its stoppage. One day later, animals were sacrificed, the colon was excised to determine its length and weight. Colon segments were examined histologically, while homogenates were used to assess relevant biochemical parameters and cytokines. Both preparations had similar qualitative effects on colon length and mass index, protected against inhibition of reduced glutathione, glutathione peroxidase and superoxide dismutase induced by DSS, against the elevation in myeloperoxidase, and protected against the elevation in TNFα and CINC-3 (inflammatory mediators). The histological changes induced by DSS were to a large extent prevented by both. The findings point to the potential usefulness of both STW 5 and STW 5-II in ulcerative colitis with only few differences in their efficacy. **References:** [1] Wagner H (2006) Phytomedicine 13:122 – 129. [2] Feinle-Bisset C and Andrews JM (2003) Curr. Treat. Options Gastroenterol. 6, 289 – 297 [3] Madisch A, Holtmann G, Plein K, Hotz J. (2004) Aliment. Pharmacol. Ther. 19, 271 – 279. [4] Wadie W,

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Skin-active natural products

SL12 Evaluation of anti-acne activity of selected Sudanese medicinal plants

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Acne vulgaris is disease related to increasing of sebum production, *Propionibacterium acnes* proliferation and inflammation. The methanol and 50% ethanol (v/v) extracts of 29 plant species traditionally used in Sudan for wound healing, bacterial and fungal skin infections and other diseases were tested *in vitro* for their potential anti-acne activity. The activities of these extracts were determined using an antibacterial assay against *P. acnes*, a lipase inhibitory assay, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay. Results showed that the extracts of *Terminalia laxiflora* Engl & Diels wood exhibited good antibacterial activity (MIC 125 μg/ml). The 50% ethanol extracts of *Abrus precatorius* L. seed, *T. laxiflora* and methanol extract of *Acacia nilotica* pods showed lipase inhibitory activity more than 70% at 500 μg/ml. The methanol extracts of *A. nilotica* (L) pods showed the best DPPH radical scavenging activity (IC_{50} 1.32 μg/ml). The best extract based on comprehensive activities was *T. laxiflora* 50% ethanol extract with better MIC value 125 μg/ml, antioxidant activity (IC_{50} 3.45) and lipase inhibitory activity (74.1%). Bioassay-guided fractionation of *T. laxiflora* resulted in isolation of five tannin related compounds such as ellagic acid, flavogallonic acid dilactone, terchebulin and gallic acid. Terchebulin showed good antibacterial activity MIC = 125 μg/ml and MBC = 250 μg/ml. Gallic acid exhibited lipase inhibitory activity with IC_{50} value of 149.3 μM, which showed strong inhibition compared with terchebulin, IC_{50} 260.7 μM. However all compounds exhibited better or equal DPPH radical scavenging activity to (+)-catechin as positive control (IC_{50} 8.23 μM). Ellagic acid and terchebulin showed the best DPPH radical scavenging activities, IC_{50} 4.86 μM and 4.90 μM respectively. This study demonstrated that terchebulin possess a potential as an anti-acne agent. **Reference:** [1] Muddathir et al. (2013) J. Wood Sci.59:73 – 79.

SL13 Development of a cosmetic plant active ingredient: sustainable and scientific considerations. The example of an ash manna tree extract *(Fraxinus ornus L.)*

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The development of a cosmetic plant active ingredient requires multidisciplinary scientific skills: agronomy, botany, phytochemistry, cutaneous biology, process engineering research. Moreover, other kinds of aspects have to be focused as social and environmental considerations. Indeed, the manufacture of a botanical ingredient requires the establishment of a supply system in order to dispose of raw plant material. The approach used by Yves Rocher's researchers for the development of their botanical active ingredients will be exposed in this presentation: from the plant discovering to the final industrialization of the ingredient. This manufacturing process can be divided in four different phases: selection of plant candidates; assessment of the project feasibility; development of the plant extract on a lab scale (cross-optimisation between phytochemistry and in vitro biological testing) and the final industrial phase scale. An example will be developed to illustrate this process: a new ash tree manna *(Fraxinus ornus L.)* extract that showed excellent results in skin nutrition. The extraction process was optimized using physical methods and natural solvents leading to a mannitol concentrated extract. New biological skin properties (in vitro evaluation) were discovered for this extract including improvement of epidermic barrier function, decrease of inflammation, and stimulation of 5 alpha reductase enzyme. The manna supply system involves a partnership and multiyear commitments of Yves Rocher with local suppliers in Sicily as well as selection and quality controls of the raw materials. This innovation contributes to maintain the crop of the ash trees as well as the traditional local manna harvest in a sustainable approach.

SL14 Tyrosinase inhibitory compounds from *Juglans sigillata* Dode

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Tyrosinase is a multifunctional copper-containing polyphenol oxidative enzyme, which is considered to be a key enzyme in melanin synthesis [1]. Some natural compounds with tyrosinase inhibitory activity of plants origin have significant potential to be applied in skin-lightening and depigmenting in the cosmetic field [2]. In this investigation, the phytochemical study, including fractionation and purification, of 70% acetone extract of *Juglans sigillata* husks led to the isolation of five low molecular weight galloyltannins and their structures were elucidated as 2,3,4,6-tetra-*O*-galloyl-β-D-glucose (**A**), 3,4,6-tri-*O*-galloyl-β-D-glucose (**B**), 1,2,6-tri-*O*-galloyl-β-D-glucose (**C**), tannic acid (**D**), and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**E**) mainly based on their spectral and chemical clues. Compounds **A-E** showed strong inhibitory activity against mushroom tyrosinase with IC_{50} values ranging from 35.27 to 76.37 uM, comparing with kojic acid which was used as a positive control with IC_{50} value of 342.14 uM. It was further found that galloyltannins **A-E** inhibited melanin production and exhibited intracellular tyrosinase activity, as well as down-regulated mRNA and protein expression levels of tyrosinase in B16F10 mouse melanoma cells. Therefore, the isolated galloyltannins from residues of *J. sigillata* may serve as potential candidates as remedy for hyperpigmentation and as skin-whitening agents in cosmetics industry. **References:** [1] Shiino M, et al. (2003) Bioorg Chem 31: 129 – 135. [2] Zocca F, et al. (2010) Bioresour Technol 101: 3791 – 3795. **Acknowledgements:** This work was supported by National Natural Science Foundation of China (31000279, 31170541), Natural Science Foundation of Tianjin City (13JCZDJC), Program for New Century Excellent Talents in University (NCET-10 – 0951), Foundation (2012IM002) of Key Lab of Industrial Fermentation Microbiology of Ministry of Education & Tianjin Key Lab of Industrial Microbiology, Tianjin University of Science & Technology, China.

SL15 Proanthocyanidins from *Rumex acetosa* L. increase the *in vitro* rate of phagocytosis of *Porphyromonas gingivalis* in murine macrophages and provide a cytoprotective and anti-inflammatory potential for modern periodontitis therapy

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Periodontitis is a chronic inflammatory disease, triggered by various bacteria. One of the major pathogens involved in the progression of periodontitis, is *Porphyromonas gingivalis*, a gram-negative anaerobic bacteria. Using multifunctional virulence factors, the bacterium is able to evade the host immune response, without inhibiting the inflammatory cascade. The persistent inflammation leads to subsequent tissue damage, which the bacteria use for nutrient aquisition. Phagocytosis by macrophages plays a crucial role in the first line innate immune defense against *P. gingivalis*. We have previously reported on the anti-adhesive effects of Rumex extract and activity against biofilm formation. Aim of the following study was the investigation of effects of Rumex extract on macrophage activity and on the anti-inflammatory potential. Acetone-water extract (7:3) from *Rumex acetosa* L. (RA1) was tested on *in vitro* phagocytosis rate of murine RAW 264.7 macrophages and the respective NO-production. The viability of the cells was controlled by MTT assay. In addition to that the release of inflammatory cytokines was

quantified by ELISA. The results show that the extract RA1 significantly stimulates the uptake of Zymosan particles (0,1 µg/mL: + 18%; 1 µg/mL: + 9% stimulation) and furthermore the phagocytosis of *P. gingivalis* (0,1 µg/mL: + 53%; 1 µg/mL: + 39%) by RAW 264.7 macrophages. No influence of RA1 was observed for NO production. In a costimulation experiment with LPS-activated macrophages, the NO production could slightly be decreased by the extract (0,1 µg/mL: -7%). Within MTT assay it was observed that RA1 shows a cytoprotective effect on the viability of the macrophages (0,1 µg/mL: + 24% cell viability). RA1 (10 µg/mL) decreases cytokine expression of IL 6 and IL 8 from *P. gingivalis* infected KB cells.

SL16 *In vitro* testing of skin regenerating potential of fungal polysaccharides and glycoproteins

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Adequate immune response to tissue damage, proliferation and functionality of endogenous cells are crucial for normal skin tissue regeneration. Modulation of immune responses and immune mediated stimulation of skin cells can be achieved by non-specific stimulants, with glycoproteins and polysaccharides being among the most potent ones. *In vitro* capability of fungal glycoproteins and polysaccharides to promote skin regeneration and wound healing was evaluated. Polysaccharide extracts from *Lentinula edodes, Ganoderma lucidum* and glycoprotein fractions isolated from *Penicillium lanoso-viride* were tested on dermal fibroblasts (DF), keratinocytes, peripheral blood mononuclear cells (MNC) and macrophage subpopulations CD 14, CD 16, CD 15/CD 56. Changes in cell proliferation were assessed by Roche xCelligence cell monitoring system. Release of cytokines was analyzed by ELISA and Luminex multiplexing technology. Results showed that all tested extracts stimulate keratinocyte proliferation, most potent stimulators being *P. lanoso-viride* glycoproteins -25% decrease in population doubling times was observed during 96 h. Glycoprotein and polysaccharide conditioned MNC cultivation medium even more promoted keratinocyte proliferation, however DF's doubling times decreased. This indicates that extracts on non-intact skin might affect skin cells also indirectly through immune cells. Analysis of cytokines in MNCs and MNC-dermal and MNC-keratinocyte cocultures showed immediate increase of TNF-α levels, however concentrations decreased gradually during 8 h. Similar secretion pattern was observed for MIP-1β. On the contrary levels of IL-10 increased continued to increase gradually. This indicates that fungal extracts possess immunomodulatory characteristics – stimulation of early inflammatory response but suppression of prolonged inflammation by induction of anti-inflammatory cytokines. Based on cytokine secretion data CD 14 macrophages were identified as the most responsive to stimulation.

SL17 Non-cellulosic glucans as inductors for differentiation of human keratinocytes

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The epidermis is a dynamic renewing structure, principally formed by highly specialized keratinocytes. During a carefully choreographed program of differentiation keratinocytes undergo complex morphological and biochemical changes. As chitooligomers were shown to stimulate cellular differentiation (1), β-D-glucans are supposed to induce human keratinocyte differentiation. The aim of the study was to identify glucans as inductors for cell differentiation, to establish an *in vitro* test system for distinct structure-activity relations and to identify the molecular targets for interaction of the glucans with the keratinocytes. Using a combination of qPCR for quantification of differentiation-specific genes, western blotting for differentiation-specific proteins and laser-scanning microscopy for verification of results on cellular differentiation we obtained two selected candidates with a high and promising potential of inducing cellular differentiation from a variety of β-glucans. Lichenan (β-1,3/1,4-glucan) from *Lichen islandicus* (L) and xyloglucan (β-1,4/1,6-glucan with defined xylose and galactose side chains) from *Tropaeolum majus* (X) each 100 µg/ml, turned out to be inductors of epidermal differentiation. qPCR analyses revealed the gene expression of differentiation related genes to be regulated in a structure-dependent manner by the test compounds. Thus L increased mRNA levels of both differentiation markers involucrin and cytokeratin 10 at an early stage, whereas X increased mRNA levels in a suqsequent stage. Besides the differences in functionality among the test compounds along with an up- and down-regulation in a time-dependent manner, there also could be found a different functionality for the positive controls compared to the test compounds. **References:** [1] Deters A, Petereit F, Schmidgall J, Hensel A (2008) N-Acetyl-D-glucosamine oligosaccharides induce mucin secretion from colonic tissue and induce differentiation of human keratinocytes. J. Pharm. Pharmacol. 60(2), 197 – 204

SL18 Skin UV-protective effect of *Microsorum grossum* extract on human dermal fibroblast

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Microsorum groβum (Polypodiaceae family), locally called "metuapua'a", are two of the most frequently fern species used in Polynesian traditional medicine. Fronds or rizhomes of this species are common ingredient of popular medicine recipes to cure various ailments. *M. groβum* frond and rizhome extracts contains phytoecdysteroids as main bioactive components such as ecdysone analogues, known to have many interesting biological activities and so considered as adaptogenic compounds. Skin UV-protective effect and mode of action of *M. groβum* extract was investigated using three ways: a transcriptomic study with c-DNA array for gene expression modulation, a stress induced premature senescence (SIPS) test on human dermal fibroblasts and cellular response experiment by activation of protein p53. The total extract of *M. groβum* up-regulates both heme oxygenase 1 and ferritin in human fibroblasts of the m-RNAs, enzymes which protect cells from oxidative stress (through the liberation of biliverdin, itself quickly converted into the antioxidant bilirubin) and which exerts several activities like the photoimmunoprotection of skin by UVA through the liberation of CO. The present work also report that premature senescence of human skin induced by repeated UV irradiations can be prevented by an ecdysteroid fraction of *M. groβum*. The activation of the protein p53 by ecdysone content of *M. groβum* is also indicative of a protective effect provided by cellular response including damage DNA repair processes. It seems therefore that extracts of *M. groβum* could protect skin against oxidative stresses and they could be used to formulate innovative cosmetic products.

SL19 Keratinocytes as effectors in allergic contact dermatitis towards sesquiterpene lactones – Different behavior of HaCaT and NHEK cells

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In allergic contact dermatitis (ACD) keratinocytes (KC) contribute to the sensitization phase as well as the elicitation of the skin reaction after hapten contact. One of the best known mechanisms of this pathological reaction is the release of pro-inflammatory mediators by these skin cells. After hapten contact such mediators are able to enhance the immunological reaction, which can lead to an impaired regulation of the immune reaction and result in a contact allergic reaction [1]. Among the many natural compounds causing ACD, sesquiterpene lactones (STL) from Asteraceae are well known. To investigate the contribution of KC to the onset of STL-related ACD by production of proinflammatory mediators, supernatants of KC cultures treated with STL were examined by ELISA for the concentration of TNF-α, IL-6, IL-8 and IFN-γ. In case of HaCaT-KC a strong increase of IL-6 as well as IL-8 was observed for most of the tested STL as well as the positive control dinitrochlorobenzene (DNCB) [2]. Most interestingly this effect could not be observed with normal human epidermal keratinocytes (NHEK) treated with STL or DNCB. Quite interestingly, however, supernatants of both HaCaT as well as NHEK increased the expression of intercellular adhesion molecule 1 (ICAM-1 or CD 54) on normal human dermal fibroblasts (NHDF). This effect was enhanced by the test compounds in HaCaT but diminished in NHEK cultures. These findings implicate a possible involvement of KC in the ACD towards STL by means of altered secretion of proinflammatory mediators. Yet the differing results in the two cell model systems are quite interesting. Further studies will have to investigate the detailed reason for this discrepancy and address the question, whether either of

those models is more reliable than the other in studies on inflammatory/immunological processes. **References:** [1] Albanesi C., Curr. Opin. Allergy Clin. Immunol., 10, 452 – 456, 2010 [2] Hoffmann MKF, Schmidt TJ, Planta Med. 78, 1138, 2012

SL20 Alkannins and Shikonins: From ancient codes to modern medicine

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There are few natural products with histories as rich as those of the enantiomeric naphthoquinones alkannin and shikonin (A/S). Their story can be traced back many centuries, where extracts from the roots of *Alkanna tinctoria* (A.t.) in Europe and *Lithospermum erythrorhizon* (L.e.) in the Orient have been used independently as natural red dyes and crude drugs with the magic property of accelerating wound healing. The first recorded use of A.t. roots is found in the works of Hippocrates and Dioscorides for the treatment of ulcers. Since then, the medicinal properties of the plant either have drifted into folklore, or been forgotten. In 1976 V.P. Papageorgiou revived the study of these plants and discovered the science behind ancient codes. The results of his experiments confirmed the wound healing, antimicrobial and anti-inflammatory properties of A.t. root extracts and was the first to identify alkannin derivatives as the active components. He developed several pharmaceutical preparations, the clinical trials of which proved their outstanding efficacy in patients with indolent ulcers, burns, wounds. These were approved by the National Organization for Medicines (Greece) (Histoplastin Red®, HELIXDERM®). Since then, our research focuses on chemistry, biology and technology of A/S. The discovery of oligomeric A/S brought new extensions in chemistry of naphthoquinones. The significant antioxidant activity and anti-*Leishmanial* action of A/S were confirmed. New clinical trials of HELIXDERM® on diabetic and indolent ulcers present impressive results. Drug delivery systems for A/S (microcapsules, liposomes, hyperbranched polymers, chimeric systems, nanofibers) have been formulated. The ancient medicinal properties claimed for A.t. and L.e. have been confirmed by scientific experimentation the last 35years. A/S are considered a class of medicines that greatly augment the modern therapeutic arsenal, confirming that natural products can be promising for the development of new pharmaceuticals.

Ethnopharmacology of African medicine

SL21 Evaluation of nigerian ethnomedical plants for anti-infective and antidiabetic properties

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There is need for more scientific justifications for many African plants used ethnomedically in treating infections and diabetes. Extracts and ashes of some of them were evaluated for these activities using standard methods while their active constituents were identified using spectroscopy. Leaves of *Jatropha tanjorensis*, *Eugenia uniflora, Bauhinia monandra, Stachytarpheta cayennensis*, *Gongronema latifolium* stem and root, and *Clausena lansium* stem bark had antihyperglycaemic actions that were due to insulin release. Insulin stimulation was reported as an unreported mechanism of their actions. Insulinotropic quercetin, isoverbascoside, 1:1 mixture of α- and β-amyrin cinnamates, and chalepin and imperatorin were the active constituents of the last four plants. HPLC confirmed their relative proportions in the active fractions, while synergism in some of the constituents and active fractions were also observed. Rutin from *B. monandra* was a pro-drug. *Murraya koenigii* was slow acting due to its insulin inhibitory carbazoles. The various plants may help in the management of either type 2 diabetes due to insulin resistance or its insufficiency. Six plants with higher activities than glibenclamide gave the hope of discovering new templates for drug development. Ashes containing "antidiabetic" trace elements had better activities than their extracts, which were attributable to their insulinotropic effects. The most active antiplasmodial compound of *G. latifolium* was lupenyl acetate. Larvicidal plants may complement anti-malarial activities in malarial control. Absence of toxic elements may confirm the safety of these herbal drugs. Their additional hepatoprotective, anti-microbial, -trichomonal, -inflammatory and trypanocidal properties may account for reduced death of African sufferers using herbal drugs singly or co-administered with orthodox drugs. Implications of these results in the management of diabetes will be presented.

SL22 Rooibos tea improves sperm concentration and functions in the rat

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Rooibos tea (*Aspalathus linearis*) grows in the Western Cape region of South Africa. Unfermented and fermented rooibos contain high levels of antioxidants which inhibit reactive oxygen species (ROS). As spermatozoa are highly susceptible towards ROS, rooibos tea may improve sperm functions. To test this hypothesis, male rats were given 2% or 5% unfermented or fermented rooibos tea as sole source of drinking for 52 days. No significant alterations were observed in body weight gain, reproductive organs weight and serum antioxidant capacity, but testosterone levels were slightly lowered. Seminiferous tubules displayed complete spermatogenesis with abundant sperm in the lumen. However, a significant decrease in tubule diameter and germinal epithelial height were observed. In the epididymides, epithelial height of caput region showed a significant increase. Drinking of unfermented rooibos significantly enhanced sperm concentration, viability and motility. Fermented rooibos also boosted sperm concentration and motility and significantly improved sperm vitality. All teas caused a small increase in spontaneous acrosome reaction which was only significant for 2% fermented rooibos. Creatinine was significantly enhanced in all treated rats, consistent with significant higher kidney weights. Activity of liver marker ALT was significantly reduced, whereas the effect on AST varied: 2% unfermented rooibos significantly decreased AST level but the 5% fermented tea increased it. In conclusion, rooibos tea significantly improved sperm concentration, viability and motility which is beneficial for male fertility and might be attributed to its high level of antioxidants. The increased level of acrosome reaction caused by rooibos may be a reason for concern as this could inhibit the fertilization process. Also, the prolonged exposure of rooibos might result in subtle structural changes in the testis. Intake of large amounts of rooibos tea may also impair liver and kidney function.

SL23 Traditional African Medicine as a source of biologically active substances inhibiting neuroinflammation

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Traditional Africa Medicine (TAM) refers to indigenous forms of healing that are practiced all over Africa. One of the most important forms of TAM is the use of herbal extracts for the prevention and treatment of diseases. Neuroinflammation has been shown to be a critical aspect of neurodegenerative disorders, including Alzheimer's disease. Here we show that extracts and bioactive compounds obtained from African plants are able to inhibit neuroinflammatory processes. Our research has shown that cryptolepine, an alkaloid of the West African shrub, *Cryptolepis sanguinolenta* (Lindl.) Schltr (Apocynaceae) inhibits neuroinflammation in lipopolysaccharide-(LPS)-activated microglia. This alkaloid has been shown to inhibit inflammatory mediator release from activated microglia through mechanisms involving NF-kB and p38 MAPK signalling. Cryptolepine also produced anti-neuroinflammatory actions in IL-1β-stimulated SK-N-SH neuronal cells. Other African plants which have been shown to exhibit varying degrees of inhibition of neuroinflammation are *Anacardium occidentale*, *Bridelia ferruginea*, *Picralima nitida* and *Capsicum* extract [1], [2]. The results presented in this talk provide a molecular basis for the potential of these African plants in neuroinflammation. **References:** [1] Olajide OA, Aderogba MA, Okorji

UP, Fiebich BL. *Bridelia ferruginea* produces antineuroinflammatory activity through inhibition of Nuclear Factor-kappa B and p38 MAPK signalling. Evid Based Complement Alternat Med 2012; 2012: 546873. [2] Olajide OA, Aderogba MA, Fiebich BL. Mechanisms of anti-inflammatory property of *Anacardium occidentale* stem bark: inhibition of NF-κB and MAPK signalling in the microglia. J Ethnopharmacol 2013; 145: 42 – 49.

Ethnopharmacology of Amazonian medicine

SL24 Establishment of a sustainable wild collection for Jaborandi (*Pilocarpus microphyllus* STAPF ex WARDLEWORTH) in the pre Amazonian Area in Brazil

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For manufacturers of natural compounds a sustainable supply is crucial. However, several plants are not that easy cultivated as their original habitat is within complex ecosystems. These cases require strategies which assure, that the natural resources are used in a way that guarantees: I. Sustainable use of Biodiversity II. Work is done with social responsibility and III. Business is economically attractive [1]. The leaves of Jaborandi are the major source for purification of the parasympathomimetic API Pilocarpine. Efforts have been made to cultivate the shrub [2], but wild collected leaves are still important because of their much higher concentrations [3], as the alkaloid biosynthesis is more influenced by environmental than genetic conditions [4]. Centroflora and Boehringer Ingelheim initiated a project with GIZ with the aim to fulfil sustainable supply criteria in the states of Piauí, Pará and Maranhão in 2011. Project outline

1. Identification and registration of collectors
2. Set up of collection centres that coordinate harvesting, packing of dried leaves and audit collectors and distributors
3. Training of collectors in:
– Harvesting techniques
– Intoxication prevention
– Drying, packaging, labelling
4. Issuing collector passports that identify collectors to rangers and authorities
5. Harvest campaigns
– Definition of harvesting period, quantities, details
– Price negotiation
– Monitoring of harvesting activities
6. Capacity building on cooperativism and associativism.
– Evaluation for continuous improvement
– Updating of legal compliance
7. Impact
– The procedure allows the crop to recover and propagate, raises the collectors' average income and provides the industry with stable supply.

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Computational methods in natural products chemistry

SL25 Pharmacophore-based identification of novel hERG channel blockers of natural origin – Development of a virtual screening workflow and experimental validation

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The human ether-a-go-go-related gene (hERG) channel plays a critical role in cardiac action potential repolarization. hERG block can lead to arrhythmia and increased incidence of sudden death. Although several drugs have been removed from the market for this reason, it has just recently been of interest to assess the potential cardiotoxic risks of botanicals. The goal of this study was to design, experimentally validate and apply a virtual screening workflow to identify novel hERG channel blockers, with focus on the investigation of natural products. A ligand-based pharmacophore model collection was developed and theoretically evaluated against databases of known hERG blockers (ChEMBL) and drug-like decoys (WDI). The seven most complementary and suitable models were then used for virtual screening of in-house and commercially available compound libraries. Fifty chemically diverse compounds, including natural products, were selected from the hitlists for bioactivity testing on *Xenopus laevis* oocytes, using a voltage clamp technique. Cells were treated with 30 μM solutions of the compounds, and 30% reduction of the peak tail hERG current was defined as the cut-off for positive blockade. This campaign identified twenty hERG blockers showing an inhibition between 32 and 79%. In summary, we have demonstrated that our virtual screening approach was successful in identifying novel hERG blockers. These experimentally validated models represent a valuable predictive tool in the assessment of potentially cardiotoxic natural compounds incorporated into a international Marie Curie project aiming at target-oriented identification and isolation of hERG channel blockers from highly consumed botanicals. **Acknowledgements:** Work supported by a Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Programme (hERGscreen). DS thanks UIBK for her position in the Erika Cremer Habilitation Program.

SL26 Temporal characteristics of a natural products in-house database

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In the era of Big Data it is necessary to transform data into knowledge so that information has high value gain. In this context, our research group AsterBioChem wishes to contribute for this Big Data in the field of natural products. A newly developed in-house database of plants from South American Asteraceae called AsterDB (*Aster*BioChem *Data*base) comprises diverse chemical structures and additional information of compounds isolated by AsterBioChem members. The AsterDB has taxonomic information, biological activities and structural information. This database can be used for chemosystematic studies, extract dereplication, QSAR and QSRR studies. In this work we describe a QSRR study involving sesquiterpene lactones from AsterDB. The chromatographic information comprises logarithm of retention factor obtained from experimental retention times using reversed phase analytical C-18 column in isocratic elution (MeOH-H_2O 1:1 and 3:7 MeCN-H_2O). The 2D and 3D descriptors were calculated using softwares PaDEL, Adriana. Code, Dragon and MOE. Pre-processing of the descriptors was carried out with the Caret's package from R. Four procedures for descriptor selection were used: genetic algorithm (GA), forward selection, best first and greedy stepwise. Artifi-

cial neural networks (ANN) with the backpropagation algorithm and partial least squares (PLS) were used as modeling tools. More than 300 models were built using different combinations of training and test sets. The best models were selected and those with overfitting and external validation with Q^2ext< 0.7 were discarded. The best model for MeCN was obtained by PLS/GA using 2D descriptors (R^2= 0.96, Q^2= 0.92 and Q^2ext = 0.91) and that for MeOH was obtained by ANN/GA using 3D descriptors (R^2= 0.91, Q^2= 0.88 and Q^2ext = 0.80). This QSRR modeling showed that AsterDB is able to transform data into knowledge for future use in dereplication of secondary metabolites from plant extracts. **Acknowledgements:** FAPESP, CAPES, CNPq

SL27 Discovery of plant anti-inflammatory biomarkers by machine learning algorithms and metabolomic studies

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NSAIDs are the most used anti-inflammatory (AI) drugs in the world. However, side effects still occur and some inflammatory pathologies lack efficient treatment. Cyclooxygenase (COX) and lipoxygenase (LOX) pathways are of utmost importance in inflammatory processes and therefore novel inhibitors for both of them are needed. Dual inhibitors on COX-1 and 5-LOX should be AI medicines with high efficacy and low side effects [1]. As AI activity of species from Asteraceae is well-known, we screened 55 leaf extracts (EtOH-H_2O 7:3, v/v) against COX-1 and 5-LOX. Among the tested extracts, 13 of them (26.6%, IC_{50} range from 0.03 – 36 μg/mL) displayed the desired inhibition. Each extract was further analysed by HPLC-HRFTMS. The data of all samples were processed employing a differential expression analysis software (MZmine 2.6) coupled to the Dictionary of Natural Products for dereplication studies. The 6,052 characteristic peaks in the extracts according to their respective AI properties were selected by genetic search (Weka 3) and 1,261 of them remained. An additional selection by decision trees J48 (Weka 3) was carried out and 11 substances were determined as biomarkers for the dual inhibition. Finally, a model to predict new biologically active extracts was built by multilayer perceptron using the biomarkers data (70% of active and non-active samples comprised the training group and 30% the test group). In summary, we developed a new and robust model for prediction of the bioactivity of natural compounds, resulting in high percentage of correct predictions (90%), high precision (100%) and low error values (mean absolute error = 0.2) as also shown in the validation test. Thus, the biomarkers of the plant extracts were statistically correlated with their AI activities and therefore can be useful to predict new AI extracts as well as their AI compounds. **Acknowledgements:** CNPq and FAPESP. **Reference:** [1] Fiorucci et al. 2001 Biochem. Pharmacol. 62:1433.

Hyphenated analytical techniques and target fishing

SL28 Who will get you there quicker, Fred Flintstone or George Jetson?

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Sequoia Sciences identifies novel chemistry from its library of structurally diverse small molecules isolated from plants. The proprietary design of this library allows for the biological screening of these compounds at optimal HTS concentrations, without non-drug-like interferences. Sequoia built this analytical process such that rapid isolation and structure elucidation of active compounds could be accomplished. Sequoia's efforts to date have been to create a high-throughput natural products chemistry program. The fictitious cartoon characters of the 1960's network television in the United States, "The Flintstones" and "The Jetsons" will be used to illustrate the ability to create a high-throughput natural products chemistry program without the bottleneck of structure elucidation being the rate limiting step. The improvement of NMR probe technology has created a tremendous sensitivity advantage for today's natural products chemist. From the early days of 5 mm NMR tubes which demanded milligrams of material, to the CapNMR Probe where one could start to realize the sensitivity gains of recording full data sets on 10's of micrograms of material, to the state of the art 1.7 mm MicroCryoProbe. The scientific strategy that Sequoia employs in order to rapidly uncover the chemical diversity contained in plant natural products for its internal cancer program will be outlined. This presentation will expand upon the ground breaking NMR tube transitions to the CapNMR probe and now the TCI 1.7 mm MicroCryoProbe. An example that was realized from this NMR probe advantage will be presented from Sequoia's cancer program. For the structure elucidation process, this advanced capillary scale NMR cryoprobe acquires complete NMR data sets on micrograms (10ugrams) of material. The MicroCryoProbe technology compliments its Sequoia's current platform technologies for high-throughput natural products research for its drug discovery program.

SL29 Metabolomic strategies in dereplication for targeted cultivation and isolation of new bioactive secondary metabolites from fungal endophytes and marine microbial symbionts

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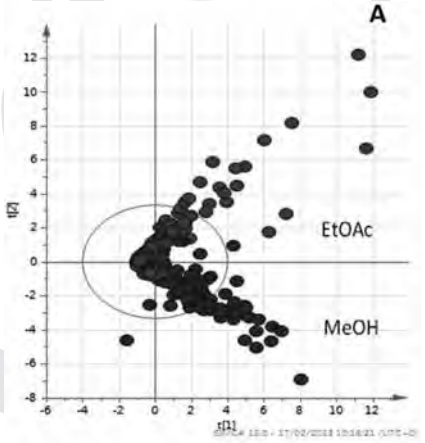
High resolution Fourier transform mass spectrometry (HRFTMS) and nuclear magnetic resonance (NMR) spectroscopy were employed as complimentary metabolomic tools to dereplicate chemical profiles of sponge-associated bacteria and fungal endophytes. The innovative strategy involved targeted cultivation and isolation of biologically active compounds. Principal Component (PCA), Hierarchical Clustering (HCA), and Orthogonal Partial Least Square-Discriminant (OPLS-DA) Analysis were used to analyze the HRFTMS and NMR data of culture extracts. The results of the statistical analysis identified and validated the best culture conditions and extraction procedure which optimized the isolation of novel bioactive metabolites. Sponge-derived actinomycetes have become a rich source of new natural products with interesting pharmacological activities. New O-glycosylated angucyclines, were isolated from the broth culture of *Actinokineospora* sp. strain EG49 which was cultivated from the Red Sea sponge *Spheciospongia vagabunda*. The structure of the compounds were determined by 1D- and 2D-NMR techniques as well as high resolution tandem mass spectrometry.



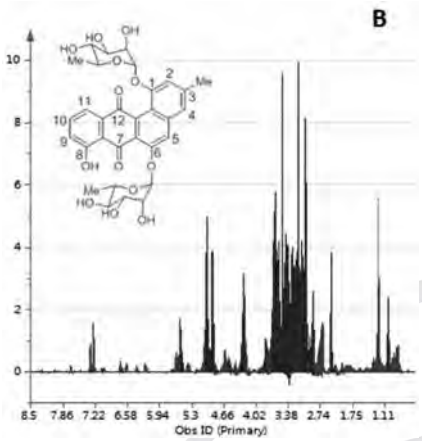


Fig. 1: A) PCA-Score scatter plot of EG49 MeOH (blue) vs. EtOAc extracts (red); B) line plot obtained from the hierarchical clustering analysis of the PCA results for EG49 extracts where the primary observed ID represents the ^{1}H chemical shifts in ppm.

SL30 State-of-the-art hyphenated technique HPLC/UV-MS/SPE/NMR interface for new approaches in phytochemical exploration of orchids

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In order to complete our investigations of the chemical diversity of orchids, a HPLC-DAD-HRMS/MS dereplication strategy combined with a hyphenated HPLC/UV-MS-SPE-NMR technique has been tested on an *Aerides rosea* Lodd. ex Lindl.&Paxt. (Orchidaceae) stem extract. Currently, main phytochemical approaches are based on several chromatographic and spectrometric techniques, like LC-DAD, LC-MS or NMR 1D and 2D. However, NMR and MS data are decoupled and a NMR analysis requires a prior total purification of some milligrams of compound of interest and consequently hundreds of grams of dried plant. The availability of raw material is often an obstacle to further analysis. Furthermore, this traditional phytochemical approach can take several months. With hyphenated LC/DAD-MS-SPE-NMR, analyses are performed on-line, directly linking the complementary nature of NMR and MS data for phytochemical analysis. This state-of-the-art technique enables a rapid identification of the constituents present in hundreds of micrograms of a simplified fraction, without any other time-consuming step of purification. Because orchids are a precious raw material, this hyphenated technique seems ideally suited. This approach allowed the characterization of nine stilbenoids in *Aerides rosea* stems. Four of them were identified by a dereplication strategy, in comparison with previous phytochemical studies of orchids [1]. The analysis of two minor fractions permitted the identification of five additional minor stilbenoids, including two phenanthrenes, two dihydrophenanthrenes and one phenanthropyran derivatives. Their structures were determined from MS and NMR (1D and 2D) data obtained only from some micrograms of each compound of interest, thanks to the capNMR probe. This equipment appears to be a great interest in Pharmacognosy field and permits a rapid on-line identification of minor natural substances, using only few quantities of plant extracts. **Reference:** [1] Simmler C. et *al.*: *PlosOne*. 2010 Oct 28;5(10):e13713.

SL31 LC-MS/MS Metabolic profiling of different Bamboo leaf extracts

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In Europe, bamboo is mainly known as ornamental plant, as a source for application in the wood and fiber industry and as an energy crop. However, a growing interest in alternative use of bamboo can be observed because this fast growing plant provides a rich natural source of promising phytochemicals and exhibits many beneficial physiological effects such as anti-inflammatory, anti-oxidant, anti-viral, anti-aging properties or prevention against cardio vascular diseases. Despite this, there is still a lack of information on the responsible secondary metabolites present in the many different bamboo species around the world. Thus, a great need for fully characterized and controlled bamboo extracts for therapeutic, cosmetic and beverage applications exists. In this study, the qualitative and quantitative chemical compositions of the leaves of morphologically heterogeneous bamboo species were determined by HPLC-MS/MS. Bamboo leaf extracts of three different bamboo genera, *Phyllostachys*, *Fargesia* and *Sasa* as well as young and old leaves were analyzed and statistical data analysis tools like principal component analysis (PCA) were applied for targeted and non-targeted metabolic profiling. Leaf extracts of different bamboo genera can easily be differentiated by targeted LC-MS/MS metabolic profiling on basis of major flavonoid composition. However no significant differences could be observed for extracts of different leaf age. Non-targeted statistical data analysis was therefore applied making use of high resolution LC-MS/MS data. On basis of non-targeted PCA analysis young leaves can be clearly differentiated from old bamboo leaves (see figure 1). Statistical data analysis results in new molecular features for differentiation. The subsequent LC-MS/MS structural characterization of relevant metabolites will be discussed. These results help to better understand physiological properties of bamboo extracts on a molecular level in cosmetical and pharmacological applications.

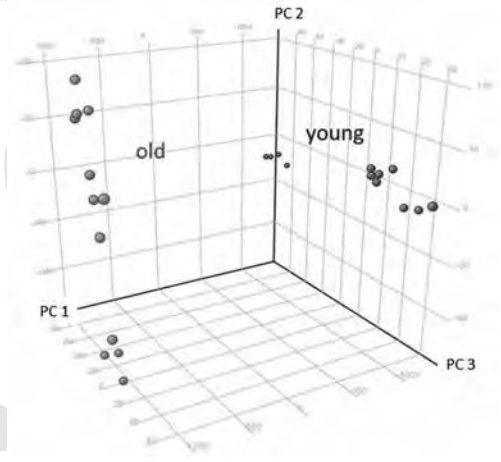

Fig. 1

SL32 Electromigration pattern of *Saponaria* saponins determine their synergistic toxicity enhancement ability of Saporin based targeted immunotoxins *in vitro* and *in vivo*

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Charge of saponin plays a crucial role in augmenting the efficacy of plant based targeted toxins [1]. The ability of only certain saponins to enhance the endosomal escape of toxins was evaluated and set into correlation

with the electrophoretic mobility. Saponins from *Saponaria officinalis* Linn, were selected as a lead owing to their tremendous augmentation potential [2]. Since saporin (Sap-3) and saponins are present in the same plant, therefore, it was also envisaged to evaluate this evolutionarily conserved principle in detail [3]. Agarose gel electrophoresis was utilized to procure pure saponin fractions with different electrophoretic mobilities, which were tested for their ability to enhance the toxicity of receptor specific targeted toxins. Five fractions (SOG1-SOG5) were isolated with a relative electrophoretic mobility of (-0.05, 0.41, 0.59, 0.75 and 1.00) and evaluated using thin layer chromatography, HPLC, and mass spectroscopic analysis. All the fractions were tested for the cellular toxicity in HER14 and MDA-MB231 cells by impedance based real time monitoring in combination with EGF targeted bacterial and plant toxins. Live cell imaging experiments with SOG4 revealed that this saponin with a specific REM of 0.59 could assist in the lyso/endosomal release of the toxic payload without affecting the integrity of plasma membrane and could lead to the induction of apoptosis. **References:** [1] A. Weng, M. Thakur, et al., (2012). Journal of Controlled release, In press [2] M. Thakur, A. Weng, et al., Electrophoresis, 32 (2011) 3085 – 3089. [3] M. Thakur, A. Weng, et al., (2011) 19 – 29.

Natural product chemistry

SL33 Parvifloranine A and B, two 11-C skeleton alkaloids from the Australian *Geijera parviflora* (Rutaceae) formed by a Mannich type reaction, inhibit the synthesis of nitric oxide

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Geijera parviflora Lindl., commonly called wilga or native willow, is endemic to eastern mainland Australia. Previous chemical investigations have identified coumarins[1 – 2] and alkaloids[2] from its leaves and fruits, and the composition of the essential oil has also been reported.[3] As part of our efforts to explore new compounds with biological activity from Australian native plants, two novel alkaloids (parvifloranine A and B), possessing an unusual eleven-carbon skeleton linked with an amino acid, were isolated from the leaves of *Geijera parviflora*. The structures of these compounds were elucidated by extensive spectroscopic measurements including 2D NMR analyses. Parvifloranine A was found to be a mixture of two enantiomers in a ratio of 1:4, based on their separation using a chiral column. The proposed biosynthetic pathway of the compounds is discussed. Parvifloranine A inhibited the synthesis of nitric oxide in LPS-stimulated RAW 264.7 macrophages with an IC50 of 23.4 μM.

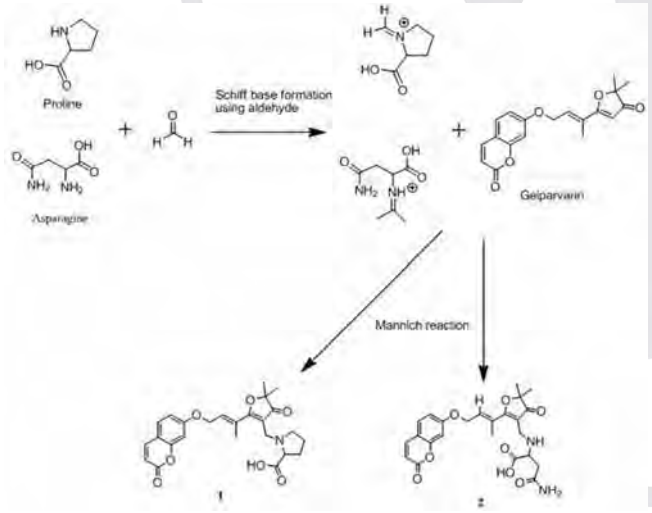

Fig. 1

Reference: [1] Lahey, F.; Macleod, J. *Aust. J. Chem*. 1967, *20*, 1943. [2] Dreyer, D. L.; Lee, A. *Phytochemistry*. 1972, *11*, 763. [3] Brophy, J. J.; Goldsack, R. J.; Forster, P. I. *J. Essent. Oil Res.* 2005, *17*, 169

SL34 Dihydroanthracenone atropodiastereomers from the endophytic fungus *Talaromyces wortmanii* with activity against multi-resistant *Staphylococcus aureus*

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Chemical investigation of the endophytic fungal strain *Talaromyces wortmannii*, isolated from the medicinal plant *Aloe vera* (Xanthorrhoeaceae), yielded three atropodiastereomeric pairs, including two symmetrical bisdihydroanthracenones, flavomannin A and its new atropisomer flavomannin B, two new asymmetrical bisdihydroanthracenones, and two new dihydroanthracenone/anthraquinone dimers. The planar structures of the new derivatives were unequivocally identified by spectroscopic and mass spectrometric analyses. The axial chirality of the biaryls was deduced from TDDFT ECD and VCD calculations, which however failed to establish the central chirality elements of flavomannin A. All six compounds exhibited antibacterial activity, predominately directed against *Staphylococcus aureus*, including even high-level (multi)drug-resistant isolates, with MIC values from 4 to 8 μg/mL for the most active compounds. Further Gram-positive genera (*Streptococcus*, *Enterococcus*, *Bacillus*) were moderately affected (MIC 32 to 64 μg/mL). Reporter gene analyses in *Bacillus subtilis* indicated induction of SOS response for some derivatives, suggesting interference with DNA structure or metabolism. Accordingly, fluorescence microscopy demonstrated defective segregation of the bacterial chromosome and DNA degradation (J. Med. Chem. 2013, 56(8):3257 – 72). The compounds showed no activity when tested against eukaryotic THP-1 cells (leukemia cell line) and BALB cells (mouse embryonic fibroblasts) (> 32 μg/mL).

SL35 Determination of configuration at C-13 of (–)-*ent*-8β-hydroxy-labdan-15-oic acid and its biotransformation by *Battus polydamas* larvae

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Battus polydamas (Linnaeus, 1758) is a specialist phytophagous insect and its larva feed on Aristolochiaceae species, of which were isolated, among other compounds, nitro derivatives, lignoids, and diterpenoids [1]. Species belonging to the *Aristolochia* genus contain insecticidal or deterrent compounds to the majority of insects, supporting the hypothesis that such compounds represent barriers to colonization by other non-adapted lepidopteran larvae [2]. Biotransformation studies by *B. polydamas* and other Aristolochiaceae-feeder have focused mainly on aristolochic acids, which have a 3,4-methylenedioxy-10-nitrophenanthrenic-1-acid carbon skeleton. In this study, *B. polydamas* larvae were fed at laboratory with leaves of *Aristolochia giberti*, and methanolic extracts of the leaves and feces were prepared by maceration. The chemical profiles of these extracts were compared by HPLC-PDA, NMR, LC-MS, and TLC. From both extracts a new labdane diterpene, (–)-*ent*-8β-hydroxy-labdan-15-oic acid (**1**), was isolated. The configuration at C-13 of **1** was established as *R* by the PGME method [3], whereas the absolute configuration of *trans*-decalin system was proposed on basis NMR spectroscopic data and in the negative signal of the optical activity observed. In addition, two new labdane diterpenes, (–)-*ent*-8β,18α-dihydroxy-labdan-15-oic acid (**2**) and (–)-*ent*-8β-hydroxy-labdan-15,18-dioic acid (**3**), possibly sequential oxidation products of **1**, were isolated from feces extract and their structures were determined by spectroscopic analyses. The biotransformation of **1** into **2** and **3** and the excretion of these compounds in the feces suggest that **1** is detoxified by *B. polydamas*. The larvae also stored compound **3**, but its ecological role is still unclear. **References:** [1] Lopes, L. M. X. et al. (2000) Res. Adv. in Phytochem. 2: 19 – 108. [2] Nascimento, I. R. et al. (2003) Pest Manag. Sci. 60: 413 – 416. [3] Yabuuchi, T., Kusumi, T. (2000) J. Org. Chem. 60: 397 – 404.

SL36 Embellicines A and B – Absolute configuration and NF-κB transcriptional inhibitory activity

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Two new secondary metabolites, embellicines A and B (**1** and **2**), were isolated from the EtOAc extract of the fungus *Embellisia eureka*, an endophyte of the Moroccan plant *Cladanthus arabicus* (Asteraceae). The structures of these new compounds were determined on the basis of extensive one- and two-dimensional NMR spectroscopy as well as by high-resolution mass spectrometry. The absolute configuration of embellicine A (**1**) was determined by TDDFT ECD calculations of solution conformers, whereas that of embellicine B (**2**) was deduced based on ROESY correlations and on biogenetic considerations in comparison to **1**. Both embellicines (**1** and **2**) are cytostatic, cytotoxic and inhibit NF-κB transcriptional activity, indicating that inhibition of NF-κB may be a possible mechanism of action of these compounds. Embellicine B (**2**), was the most active compound encountered in this study (0.21 μM) and acts at nanomolar concentrations without affecting tumor microenvironment (J Med Chem. 2013, 56(7):2991 – 9). Furthermore, when tested towards several pathogenic microorganisms, **1** and **2** were inactive which indicated their selective cytotxicity.

SL37 Chemical constituents from the roots of the thai medicinal plant *Strophioblachia fimbricalyx*; their biological activities and proposed biosynthesis

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Strophioblachai fimbricalyx Boerl. (Euphorbiaceae) is a native plant species in northeast Thailand and it is the only one species of the genus Strophioblachia found in Thailand. The decoction of this plant is used in Thai traditional medicine for migraine treatment [1]. The roots of *S. fimbricalyx* in combination with other medicinal plants were traditionally used as an aqueous decoction to treat fevers while fermented liquor of its roots together with other medicinal plants has been used to treat cancer [2]. Chemical constituents from the roots of *S. fimbricalyx* collected from northeast Thailand were first reported by our group in 2009 and the known compound, trigonostemone (**1**), together with three new compounds, 9-*O*-demethyltrigonostemone (**2**), 3,6,9-trimethoxyphenantropolone (**3**) and 4,6,9-trimethoxyphenantropolone (**4**) were isolated [3]. Compound **2** showed interesting cytotoxicity against NCI-H187, KB and MCF7 cancer cell lines while **3** and **4** exhibited reduced cytotoxic activity. Compounds **2** and **3** also displayed *in vitro* antiplasmodial activity against *Plasmodium falciparum* (K1, resistant strain). In our continued search on bioactive compounds from the roots of *S. fimbricalyx*, fimbricalyx A (**5**), a novel phenanthrenone derivative having a rare 2*H*-benz[*e*]inden-2-one substructure, was isolated. Its cytotoxicity and X-ray crystallographic structure were published recently [4]. From our present studied, eight new compounds, fimbricalyxs B-D (**6-8**), fimbricalyx anhydrides A and B (**9-10**) and fimbricalyxlactones A-C (**11-13**), have been isolated. The structures of the new compounds were elucidated on the basis of their spectroscopic data and confirmed by single-crystal X-ray crystallographic analysis. Among the new compounds, **6** exhibited potent antiplasmodial activity with an IC_{50} value of 0.019 *m*M. A possible biosynthesis of **5** and **7 – 10** via a common phenanthrenone precursor is proposed. **References:** [1] Kaewkrud, W., Otsuka, H., Ruchirawat, S., Kanchanapoom, T. J Nat Med. 2008, 62, 124- 125. [2] Chuakul, W., Saralamp, P., Boonplean, A. Thai J Phytopharm. 2002, 9, 22 – 49. [3] Seephonkai, P., Sangdee, A., Bunchalee, P., Pyne, S. G. J Nat Prod. 2009, 72, 1892 – 1894. [4] Seephonkai, P., Pyne, S. G., Willis, A. C., Lie, W. Tetrahedron Lett. 2013, 54, 2085- 2088.

SL38 *Erythrina* alkaloids achievements and perspectives

Soto-Hernandez M[1], Garcia-Mateos R[2], San Miguel Chavez R[1], Kite G[3]
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The genus *Erythrina*, member of the Fabaceae family contains 115 species with wide phorphological variation and ecological diversity. Worldwide the major distribution of *Erythrina* species is in southern Mexico and Central America. The genus has been studied in different points of view since ornamental or culinary uses until ethnomedical and chemical studies. This is because contains compounds as flavonoids, isoflavonoids, alkaloids, tripsine inhibitors, hemaglutinins and saponins in different parts of the plants. The bark, seeds and flowers have been intensily screened to this kind of compounds and the alkaloids and isoflavonoids have received special attention because their structure and biological activity e.g. antimicrobials or calm agents. Due to the increasing attraction and rapid extention of this field, the *Erythrina* alkaloids have been regurlarly reviewed concerning occurrence, structure, analytical and spectral properties, biosynthesis, total synthesis and biological activities. The present contribution will give an overview of the studies done in our laboratory and elsewhere of the *Erythrina* alkaloids from 1994 to date covering source, structure, analytical/spectral data, biosynthesis and finally a short review of their biological activity. **References:** [1] Garin-Aguilar, et al 2005, Phytochem Anal. 16, 302 – 306 [2] Garcia-Mateos, et al 2000 Pharm Pharmacol. lett. 10, 34 – 37. [3] San Miguel Chavez, et al 2007, Phytochem Rev. 6, 167 – 173.

SL39 Alkaloid content of *Rauvolfia nukuhivensis*: genetical discrimination and new putative biosynthesis pathway

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Rauvolfia nukuhivensis (Apocynaceae), an endemic and endangered species grown in Marquesas archipelago, is currently used as traditional medicinal plant for genetical ailment treatment. The structures of the major constituents in *R. nukuhivensis* bark extract were investigated by using spectroscopic means (UV, IR, HEIRMS, NMR). The study of alkaloid content of *R. nukuhivensis*, disclosing various new chemical structures, led to the identification of several indole alkaloids belonging to different skeletons such as the sarpagan or ajmalan types as well as quinolizinium derivatives[1]. The co-occurrence of such sarpagan and ajmalan derivatives raises questions on their biosynthesis. With regard to these results, we propose a new putative biosynthesis pathway which may explain the co-occurence of such various compounds in *R. nukuhivensis*. Furthermore, as the *Rauvolfia* genus is well distributed in the Pacific region, we carried out a phylogenetic study (using DNA barcoding method) of Rauvolfia species, endemic or indigenous in Oceania, aiming to a better understanding of their chemodiversity. Actually, some metabolomes from *R. nukuhivensis* occur only in few species such as flavopereirine analogues found in *R. semperflorens* var. semperflorens or sandwicine derivatives found in *R. sandwicensis*, which may favor the biosynthesis of key biomarkers compounds. **Reference:** [1] Martin N.J., Prado S., Lecellier G., Thomas O. and Raharivelomanana P. " Nukuhivensiums, indolo [2,3-α] quinoliziniums from the Marquesan plant *Rauvolfia nukuhivensis*", *Molecules*, 2012,17: 12015 – 12022

SL40 Swartzia simplex a source of new antifungal compounds

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Invasive fungal infections have dramatically increased over the last 20 years and they became a major cause of nosocomial infections in developed countries making urgent the need of new antifungal drugs [1]. In this way, a natural resource represents an interesting source of new active compounds. The dichloromethane extract of the root bark of *Swartzia simplex* (Fabaceae) presented an interesting antifungal activity against *Candida albicans* in a bioautography assay [2]. In order to isolate the active compounds, bioguided isolation was undertaken using HPLC-microfractionation in 96 well plates and agar-overlay bioautography to localize the active compounds in the HPLC-PAD metabolite profiling of the crude extract. The analytical HPLC-PAD conditions were geometrically transferred to a preparative medium pressure chromatography (MPLC-UV) by chromatographic calculations for the efficient isolation of the active compounds at the milligram scale in one step. Using this approach ten compounds were isolated, six of them are new natural products. The structures of the isolated compounds were elucidated by classical spectroscopic methods including UV, 2D NMR and HR-MS. MIQ of the actives compounds were obtained and compare with others natural antifungal compounds [3]. **Acknowledgements:** SNF grant CR2313 – 143733 to J.L.W. and EFQ. **References:** [1] L. Ostrosky-Zeichner, A. Casadevall, J. N. Galgiani, F. C. Odds, J. H. Rex. Nat Rev Drug Discov 2010; *9* (9), 719 – 727. [2] Q. Favre-Godal, E.F. Queiroz, D. Sanglard, J.-L. Wolfender. Planta Med 2012; 78-PD 159. [3] Q. Favre-Godal, E.F. Queiroz, J.-L. Wolfender. JAOAC 2013; in press.

SL41 Targeted isolation of induced and bioactive metabolites from fungal co-cultures

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The prevalence of *Fusarium* spp. as causative agent of onychomycoses is rising and *Fusarium* spp. as well as other non-dermatophyte fungi appear to be insensitive to systemic standard treatment [1]. Hence, new antifungal agents active against *Fusarium* spp. are needed. In a large screening [2], different plant and human pathogenic fungi were co-cultured with *Fusarium* spp. in Petri dishes. Few fungi were able to keep *Fusarium* at bay, among them the Basidiomycete *Hohenbuehelia reniformis.* An induction of red pigments was visually observed in the co-culture experiments but was not particularly highlighted among the metabolites revealed by UHPLC-TOFMS-based chemometrics. Nevertheless, using a specific UHPLC-UV analysis, it could be demonstrated that several pigments were upregulated by *Fusarium* sp. upon co-culture. On the other hand, MS-based chemometrics revealed upregulation of metabolites produced by *H. reniformis*. Extracts of the mycelium of fungi grown in Petri dishes contain mainly saccharides as confirmed by NMR of the total extract. Prefractionation with the resin HP20SS was successfully applied to separate secondary metabolites from the saccharides. For the co-culture of *H. reniformis* and *Fusarium* sp., an enriched – and sugar-free – prefraction showed activity against human pathogenic *Fusarium* sp. Preliminary identification of this fraction's metabolites was done by dereplication of the high resolution MS data. These compounds are only present in small amounts (< 1% of total extract), thus co-cultures were produced on solid media at a large scale.

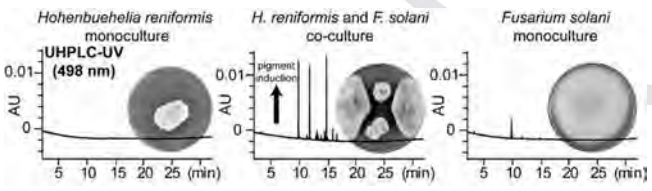

Fig. 1

The analytical strategy for the isolation of *Fusarium* pigments and anti-*Fusarium* constituents from fungal co-cultures on solid media are presented. **References:** [1] F. Baudraz-Rosselet, C. Ruffieux, M. Lurati, O. Bontems, M. Monod, *Dermatology* 2010, 220(2), 164. [2] S. Bertrand, O. Schumpp, N. Bohni, A. Bujard, A. Azzollini, M. Monod, K. Gindro, J.-L. Wolfender, *J. Chrom. A* 2013, doi: 10.1016/j.chroma.2013.01.098.

SL42 Identification of quinolactin alkaloids from fungi associated to Brazilian red algae *Dichotomaria marginata* by LC-PDA-MS and LC-MS^n

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Fungi isolated from marine organisms have been shown to produce several interesting secondary metabolites with important biological activities. Such chemical diversity may be associated to environmental stress conditions and may represent an important source of NCE for bioprospection. Quinolactins belong to a rare fungi-alkaloid class with a unique N-methyl-quinolone moiety fused to a lactam ring and present several bioactivities[1]. Fungi strain Dm1 was isolated from red alga *Dichotomaria marginata*, collected from Brazil SE coast, and was grown in sterile rice solid media at 26°C [2], which was then extracted with MeOH. The MeCN fr. from the MeOH extract was chromatographed over Sephadex LH-20 and fr. 4 afforded quinolactin (QL) alkaloids B1, B2 and A, whereas fr. 5 afforded quinolactin D1 after purification by HPLC-DAD. Structural determination of pure compounds was based on HRMS, UV, and NMR spectral analyses, in addition to comparison with literature data and Antimarin® databank. UV data indicated the presence of similar chromophores with λ_{max} at *ca.* 247 and 320 nm. HRMS and tandem MS analyses using both negative and positive ion modes for the isolated compounds indicated their molecular formula and structural features, as for QL B1: $C_{15}H_{16}O_2N_2$ [M+H 257], which showed one fragment at *m/z* 214 [-CHNO]; QL B2: $C_{15}H_{16}O_3N_2$ [M+H 273], with product ions at *m/z* 230 [-CHNO·] and *m/z* 186 [-C_4H_9NO·]; for QL A: $C_{16}H_{18}N_2O_2$ [M+H 271], which presented one ion at *m/z* 214, due to loss of fragment (-C_4H_9) from the molecular ion; and for QL D1: $C_{16}H_{18}N_2O_3$ [M+H 287], with product ions at *m/z* 186 [-CHNO] and *m/z* 230 [-C_4H_9]. Such data suggested fragmentation proposals, e.g. for Quinolactin B1 (Fig. 1), which confirmed the structures of the isolated quinolactins, and may represent an important contribution for the sustainable exploration of marine biodiversity.

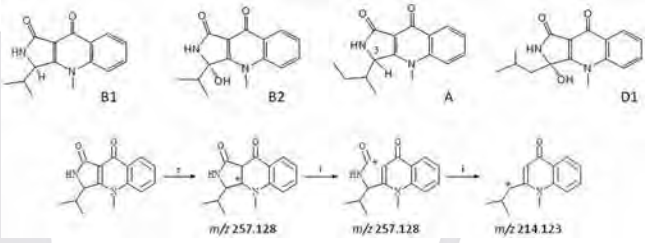

Fig. 1: Quinolactin alkaloids from endophytic fungi Dm1 obtained from red alga *Dichotomaria marginata* and fragmentation proposal for Quinolactin B1

References: [1] Cardozo, KHM et al. Comp Biochem Physiol C Toxicol Pharmacol 2007, **146**, 60. [2] Silva, GH et al. Phytochem. Lett. 2010, **3**, 164.

SL43 Converting the molecular framework of medicinal plant constituents: a fermented *Mercurialis* extract with wound-healing activity

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Aqueous fermentation of medicinal plants represents an interesting option to obtain microbiologically stable extracts. Fermented extracts from dog's mercury (*M. perennis* L.) have recently shown immunomodulating activity on the expression of cytokines like IL-1β, TNF-α, IL-8 and PGE-2 *in vitro*, results which may explain the woundhealing effect of *Mercurialis* remedies [1]. In the current study, a closer investigation on the

conversion of plant constituents during the production of a fermented extract from *M. perennis* was performed. Profiling of dichloromethane extracts showed three novel alkaloid metabolites **2**, **3**, **4** which were fully characterized by GC/MS, NMR as well as by total synthesis. These compounds may be formed by scission of the molecular framework of the dimeric alkaloid structure **1**, genuinely found in the fresh *M. perennis* extract. Furthermore, the biotransformation of the depsides phaselic and mercurialis acids were studied in the aqueous extract. Both constituents showed a second order degradation kinetics as monitored by HPLC. Several novel formed constituents like dihydrocinnamic acids, ethyl phenols and 2-hydroxy acids were also detected, the latter presumably representing degradation products from depsides and amino acids [2]. The results obtained may help to understand the complex chemistry proceeding during the fermentation of a medicinal plant extract. **References:** [1] P. Lorenz, C. Beckmann, J. Felenda, U. Meyer, F. C. Stintzing, *Z. Phytother.* 34: (2013). [2] P. Lorenz, J. Conrad, F. C. Stintzing, *Chem. Biodivers.*, DOI 10.1002/cbdv.201200424.

SL44 Monitoring metabolites production and cannabinoids analysis in medicinal Cannabis trichomes during flowering period by ^{1}H NMR-based metabolomics

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Cannabis trichomes are known as the main site of cannabinoids production, the responsible compounds for most biological activities of the plant. This study reports ^{1}H NMR based-metabolomics and cannabinoids analysis of trichomes of 4 medicinal *Cannabis* varieties, Bediol, Bedica, Bedrobinol, and Bedrocan, in order to investigate cannabinoids production and metabolites profiles of the trichomes during the last 4 weeks of flowering period. Analysis of ^{1}H NMR spectra revealed totally 6 identified cannabinoids in the chloroform extracts, THCA (1), CBDA (2), CBCA (3), CBGA (4), THC (5) and CBD (6), and 20 compounds in the water extracts including sugars, amino acids, and other acidic compounds. Quantification analysis using ^{1}H NMR method showed that production of total cannabinoids of Bedrocan and Bedica trichomes increased from week 5 (Bedrocan: 63.01 mg/g) till week 7 (Bedrocan: 111.04 mg/g) and then decreased at week 8 (Bedrocan: 79.76 mg/g). Meanwhile cannabinoids production of Bedrobinol and Bediol trichomes increased during the monitoring time. Different metabolites profiles within trichomes varieties were revealed by PLSDA models of metabolomics. Important differential metabolites in this discrimination were THCA (1) and CBDA (2) on the chloroform extracts, and were asparagine, choline, fructose and glucose on the water extracts. Furthermore PLSDA classified the trichomes of every variety based on their harvested weeks. THCA (1) was found as an important discriminant compound in the chloroform extracts of every variety. Meanwhile, threonine, asparagine and fructose were detected as differential metabolites in the water extracts of every variety. This study indicated that *Cannabis* tricomes during flowering period produced metabolites, particularly cannabinoids in different amounts depending on the time and the plant variety. Furthermore it is the first report for monitoring metabolites production in plant trichomes using ^{1}H NMR-based metabolomics.

SL45 Meliaceae plants and vector control of malaria: Larvicidal studies of *Ekebergia senegalensis* A Juss and *Cedrela odorata* Linn

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Background: Plant species are known to produce metabolites of value used as pest control that target specific organisms. Two Meliaceae plants identified and tested for their larvicidal activity against the fourth instars larva of *Anopheles gambiae,* the primary vector of malaria in sub-Saharan Africa. **Methods:** Dried powdered leaves of these medicinal plants were extracted by maceration in methanol. The most active extracts from *Ekebergia senegalensis* and *Cedrela odorata* were fractionated into hexane, chloroform and ethyl acetate solvents by liquid-liquid partitioning. Larvae of *A. gambiae* were collected. Toxicity was evaluated by exposing 4th instar larvae to different concentrations (62.5 – 1000 µg/mL) of extracts, larval mortality and LC_{50} values were determined after 24 h. **Results:** The hexane fraction of *E. senegalensis* displayed an absolute mortality at 0.63 mg/mL with an LC_{50} value of 0.81 mg/mL; also hexane soluble fraction of *C. odorata* had inhibitory toxicity on mosquito larvae with an LC_{50} value of 0.83 mg/mL. Results were compared to those of larvae exposed to N,N-diethyl-3-methylbenzamide (1.09 mg/mL), the reference insecticide and untreated groups. The hexane fractions of both plants displayed good activities compared to the reference insecticide. *Ekebergia senegalensis* extract had a very impressive larvicidal activity recording the highest mortality rate when exposed to the larvae. **Conclusion:** Though a battery of plants from different families have been reported as useful larvicides on *A. gambiae*, only a few botanicals have moved from the laboratory to field use. *Ekebergia senegalensis* crude extract showed promising activity in mosquito control, commercial utilization should be feasible, and contribute significantly to malaria control.

SL46 Scaled up countercurrent chromatography separation of secondary metabolites from *Schinus therebinthifolius* Raddi

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Schinus therebinthifolius Raddi (Anacardiaceae) is a tree of medium size native to South and Central America, whose fruits known as pink pepper, are widely used for cooking. In folk medicine, it has been used to treat ulcers, arthritis, as well as in leprosy therapy. Previous phytochemical investigations have described the isolation of polyphenols, fatty and terpenoid acids[1]. Countercurrent chromatography (CCC) is a liquid-liquid partition chromatography in which the stationary liquid phase is retained in the apparatus without the use of a solid support, while the mobile phase is pumped through the coil. Scaled up CCC was performed to isolate metabolites from a CH_2Cl_2 extract of *S. therebinthifolius* berries. Evaluation of parameters was done on an analytical Mini-DE centrifuge (17.4 mL; 0.8 mm i.d.) using heptane-EtOAc-MeOH-H_2O 6:1:6:1 (v/v/v/v) as the solvent system. Sample concentration (25, 50, 100, 125 mg/mL), volume (2.5% and 5% of coil volume) and flow-rate (0.5 and 1.0 mL/min) were studied. After systematic optimization, a linear scale-up was calculated to Spectrum-DE (143.5 mL; 1.6 mm i.d.) and to Midi-DE (912.5 mL; 4.0 mm i.d.) centrifuges, keeping the same *g* force value by adjusting the rotational speed. Detection of all runs was performed by TLC and UV (λ 210 nm). Results indicated that the scale-up settings gave good chromatographic resolution, with phase retention of about 75%. The optimal concentration value of 100 mg/mL, was kept in all experiments. All CCC runs yielded directly the isolation of two pure major triterpene acids, identified as 3β-masticadienolic acid and masticadienolic acid (Figure) by NMR and MS data[2]. Other minor compounds were also enriched and further purified. Identification of these is in progress. In conclusion, a CCC purification method was developed, optimised and scaled-up to increase throughput 130 times, whilst maintaining the run duration and separation efficiency

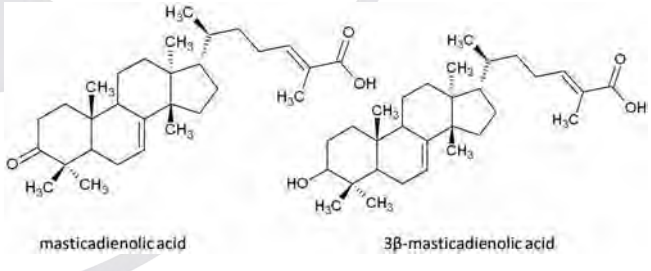

Fig. 1

References: [1] *J. Diet. Suppl.* 5 (2008) 349 [2] *Planta Medica* 42 (1981) 268.

SL47 Melanogenesis acceleratory activity of synthesized quercetin derivatives and the structure activity relationships

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Melanogenic acceleratory effect of *Helminthostachys zeylanica* root extract was examined using murine B16 melanoma cells and the potent active compounds were identified. Two quercetin glycosides namely 4'-*O*-β-D-glucopyranosyl-quercetin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside **3**, 4'-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-quercetin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside, and three ugonins, ugonin J, K, and L were isolated from *H. zeylanica* root extract by a series of chromatography. In order to elucidate the correlation of structure activity, ten quercetin glycosides included **3** and three quercetin derivatives were synthesized using rutin as a starting material. The identification of isolated compounds from *H. zeylanica* root extract and the synthesized quercetin glycosides were confirmed by NMR, MALDI or UPLC-TOFMS, IR, UV spectrum, and specific optical rotation. Among the quercetin glycosides, quercetin-3-*O*-β-D-glucopyranoside**1**, quercetin-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside **2**, and **3** showed intracellular melanogenesis acceleratory activity and 3-O-methylquercetin showed extracellular melanogenesis acceleratory activity in a dose dependent manner. These compounds showed no effect for the mushroom tyrosinase activity which catalyzes the rate limiting reaction of melanogenesis. Therefore they may have an effect on a gene expression relating to melanogenesis in melanoma cells.

Plant phenols: structures, analytics and activities

SL49 Catechol is a bioactive metabolite of Willow bark

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It has recently been shown in a bioassay guided fractionation of a Willow bark extract (Salicis cortex, *Salix spec.*, Salicaceae) that not the isolated compound salicortin itself but its degradation product catechol was responsible for an anti-inflammatory effect *in vitro*. To investigate if this *in vitro* effect has impact on the *in vivo* situation, a pharmacokinetic study was performed in humans with eight healthy volunteers. After oral administration of a Willow bark extract corresponding to 240 mg salicin, venous blood samples were taken up to 24 hours, and serum was analyzed by HPLC-DAD after processing with glucuronidase and sulfatase. Mean peak concentrations of 13.3 µM catechol were observed after 1.2 hours. No free catechol could be detected without enzyme processing and the predominant phase-II-metabolite was catechol sulfate. To exclude that the detected catechol in the human serum samples was not solely absorbed as a genuine compound of the Willow bark extract, pure salicortin 100 mg/kg b.w. was orally administered to Wistar rats. In this study, catechol could definitely be proved as a metabolite of salicortin in serum with a c_{max} of 13.0 µM after 0.5 h. These findings indicate that not salicortin *per se* but rather catechol, which is one of its active metabolites reported in the literature to have anti-inflammatory properties, contributes towards the overall ant-inflammatory and analgesic efficacy of Willow bark.

SL50 Neurogenesis-inducing flavonoids: A structure-activity study

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Diseases like Alzheimer's or Parkinson's and also ischemic incidences ("strokes") are characterized by a loss of neurons due to cell death, resulting in functional deficits. Since the discovery of multi-potent neural stem cells (NSCs) present in the adult human brain (1), considerable interest has arisen in the possibility of regenerative therapies involving exogenous compounds to target differentiation in NSCs. Searching for natural compounds which can induce neurogenesis to NSCs, prenylflavonoids called our attention, since they can cross the blood-brain barrier (2,3). Hops mostly known from brewing industry, is an abundant source of prenylflavonoids. Accordingly we did an activity-guided fractionation of a commercial hops extract. This fractionation led to a potent neurogenesis-inducing pyranoflavonoid, which showed higher activity than retinoic acid, a well known differentiation factor and other flavonoids known from literature. Moreover, it promoted neurite outgrowth, displayed neuroprotective activity and shows neuronal lineage-specific effects (4). We identified the structural characteristics responsible for the neurogenesis-inducing effects of prenylflavonoids, comparing the activity of some side chain-open and ring-closed derivates. Testing of derivatives with structural changes to the pyranoring confirmed the importance of this structural characteristic. Furthermore structural changes on A-ring as well as on B-ring of prenylflavonoids showed a specific influence on until now unknown targets. **References:** [1] Gage FH, Science 2000, 287, 14 33 [2] Butterweck V. *et al.*, J. Pharm. Pharmacol. 2007, 59, 549 – 553 [3] Rad M. *et al.*, Brit. J. Clin. Pharmacol. 2006, 62, 288 – 296 [4] Aigner L., Riepl H., Urmann C., 2012 WO2012172090 A1

SL51 Sub-lethal concentrations of carvacrol (from oregano) inhibit bacterial quorum sensing and formation of biofilms

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The formation of biofilm by bacteria confers resistance to biocides presents problems in both medical and veterinary clinical settings. Here we report the activity of carvacrol, one of the major antimicrobial components of oregano oil, on the formation of biofilms and it's activity on existing biofilms. Carvacrol was able to inhibit the formation of biofilms of *Chromobacterium* violaceum, *Salmonella* enterica subsp. Typhimurium DT104, and *Staphylococcus* aureus, while it showed no effect on formation of Pseudomonas aeruginosa biofilms. This inhibitory effect of Carvacrol was observed at sub-MIC concentrations (< 0.5 mM) where no effect was seen on total bacterial numbers, indicating that carvacrol's direct antimicrobial effect was not causing the observed inhibition of biofilm formation. In contrast carvacrol had (up to 8mM) no or very low activity against existing biofilms of the bacteria described, showing that formation of the biofilm also confers protection against this compound. Since quorum sensing is an essential part of biofilm formation, the effect of carvacrol on quorum sensing of Chromobacterium violaceum was studied. Sub-MIC concentrations of carvacrol reduced quorum sensing at concentrations coinciding with carvacrol's inhibiting effect on biofilm formation (Fig 1). These results indicate that carvacrol's activity in inhibition of biofilm formation could be linked to its activity on quorum sensing.

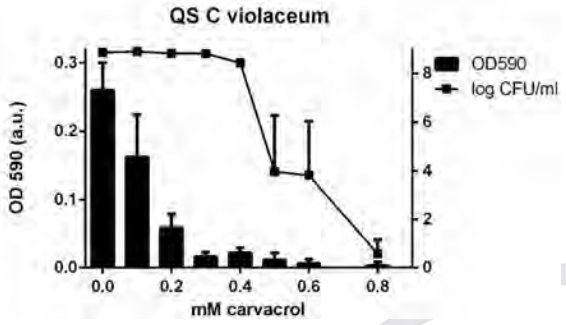

Fig. 1: Carvacrol inhibits quorum sensing (QS) in C. violaceum at the same concentrations at which it deters biofilms.

SL52

Influence of gut flora-derived ellagitannins' metabolites- urolithins on production and release of pro-inflammatory factors from stimulated neutrophils in the context of cardiovascular disease prevention

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Ellagitannin-rich products were proven to have beneficial influence on cardiovascular system [1 – 2]. Due to not well-established bioavailability of ellagitannins nowadays their gut flora derived metabolites- urolithins become a target in research of factors responsible for clinical effects. Because of the important role of neutrophils in the development of pathologies in cardiovascular system [3], the influence of urolithins A, B and C on their pro-inflammatory functions was tested. Urolithin B at concentration of 20 μM showed significant inhibition of IL-1β, IL-8 and MMP-9 production induced by LPS (26.5 ± 6.2, 31.6 ± 1.7, 49.6 ± 7.7% respectively). Urolithin C was the only active compound towards inhibition of elastase release from cytochalasin A/f-MLP stimulated neutrophils. At concentration 5 and 20 μM urolithin C decreased the elastase level by 39.0 ± 6.1 and 66.6 ± 2.9% respectively. Myeloperoxidase release was strongly inhibited by urolithin A and C (at 20 μM by 46.7 ± 4.6 and 63.8 ± 3.1% respectively). Urolithin A was the strongest ROS release inhibitor both in f-MLP and PMA stimulated neutrophils. At the concentration of 1 μM caused ROS level decrease by 42.6 ± 8.9 and 53.7 ± 5.4% respectively. Obtained results indicate, that urolithins can specifically modulate inflammatory functions of neutrophils, and thus at least partially explain observed beneficial effects of ellagitannin-rich food products, nutraceuticals and medicinal plants on cardiovascular system.

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SL53

Chemical and biological similarities between root extracts from Phak paem (*Acanthopanax trifoliatus*) and Siberian ginseng (*Acanthopanax senticosus*)

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Extracts from the roots of Phak paem (*Acanthopanax trifoliatus*) collected from 3 different provinces in Thailand, i. e., Chiang Mai, Nakhon Ratchasima and Pathum Thani and extracts from the roots of Siberian ginseng (*Acanthopanax senticosus*) purchased from China and authentic Siberian ginseng roots were prepared by decoction and refluxing with 75% ethanol. All extracts were tested for *in vitro* antioxidant activities using DPPH scavenging assay, FRAP assay and TBARS method. Aqueous and 75% ethanolic root extracts from Phak paem collected from Nakhon Ratchasima (ATS2D and ATS2R, respectively) exhibited stronger DPPH scavenging activities than others with EC_{50} of 43.34 ± 1.22 and 33.78 ± 0.70 μg/ml, respectively. All extracts showed no difference in reducing powers. Extracts from Siberian ginseng showed higher effects to inhibit lipid peroxidation. Phytochemical analysis by HPLC-DAD showed that root extracts from Phak paem and Siberian ginseng had similar chromatographic fingerprints. The main constituents identified by comparison of retention times and absorbance spectra were polyphenolic compounds of caffeoylquinic acid derivatives including chlorogenic acid, cynarin, 3,5-dicaffeoylquinic acid, 1,4-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid which also showed antioxidant effects. Quantitative analysis by HPLC revealed that ATS2D and ATS2R contained higher active constituents than those collected from other provinces, the phenolic contents were 4.80 ± 0.02 and 8.94 ± 0.03 g in 100 g extracts, respectively, similar to phenolic contents in aqueous and 75% ethanolic root extracts from authentic Siberian ginseng roots which were 4.97 ± 0.14 and 11.63 ± 0.08 g in 100 g extracts, respectively. The results suggest that the roots of Phak paem have potential in chemical and antioxidant properties similar to Siberian ginseng which could be beneficial for further pharmaceutical purposes. However, harvesting areas also play an important role to the quality of raw material.

Quality control methods for medicinal plants, extracts and isolated natural products

SL54

Quality control of phytomedicines – is the herbal medicine industry facing a crisis?

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The enormity of the herbal medicines industry is emphasised by the fact that 80% of the world's population use herbal medicines. However, due to country-specific legislation (or lack thereof) herbal medicines are often not closely regulated and quality and safety cannot be guaranteed. Herbal medicines are notoriously phytochemically complex mixtures, complicating the of quality control process which is a daunting and intricate task. The quality of herbal medicines is assessed both qualitatively through species authentication as well as quantitatively through the quantification of specific biomarkers which are used to determine the quality of raw materials and products. Established, methodical processes are necessary to standardise herbal medicines to produce consistent and reproducible products. Unfortunately the industry remains plagued by the unscrupulous practices of some suppliers and producers of herbal medicines. The quality of a wide range of commercial products (n = 100) was assessed using various analytical techniques such as single point vibrational spectroscopy, hyperspectral imaging, HPTLC and LC-MS etc. in combination with chemometric data analysis where appropriate. This paper suggests simple and practical quality assurance protocols for medicinally and commercially important plant species. It will demonstrate the effectiveness of these methods to solve significant practical problems such as discriminating between closely related plant species (e. g. *Pelargonium*, *Harpagophytum* and *Sceletium* spp.), uncovered alarming analytical results where the claimed active ingredients were not detected at all (e. g. *Hoodia* and Ginseng spp.), demonstrated that the raw material does not meet set Pharmacopoeial standards (e. g. *Harpagophytum*) and revealed several errors in labeling and packaging information. The advantages and limitations of various analytical techniques including the novel application of hyperspectral imaging will be highlighted.

SL55

Analytical and active markers for quality assessment of *Leonurus cardiaca* and *L. japonicus*: RP-HPLC, HPTLC, and ^{1}H-qNMR approaches for the determination of defined phenolic and N-containing constituents

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Officinal European *L. cardiaca*, East Asian *L. japonicus*, and South African *Leonotis leonurus* are traditionally used for cardiovascular, gynecological, and neurological disorders. Nevertheless, a phytochemical assessment as a basis for their quality control and comparison amongst them has not yet been reported. A RP-HPLC method was newly developed for the quantification of leonurine. Only the Nucleodur C 18 Pyramid column (RP-phase, polar endcapping) yielded stable retention times. No leonur-

ine could be detected in any sample of *L. leonurus* or *L. cardiaca* despite numerous literature claims including official assessment reports (EMA/HMPC 2010) that name it as an active constituent. Surprisingly, *L. japonicus* fruits (Chin. Ph.) did not contain any leonurine, either, which was thus identified as a specific taxonomic marker with known pharmacological activity as it was detected in every sample of *L. japonicus* aerial parts. Furthermore, a novel RP-HPLC method for the simultaneous quantification of twelve phenolics was developed. Ferulic acid was found in every sample of every drug. Lavandulifolioside and verbascoside were not present in any sample of *L. japonicus*, but in every sample of the aerial parts of *L. cardiaca*. Lavandulifolioside was firstly found in *L. leonurus*, just as isoquercitrin, which was also detected in *L. cardiaca* but not in *L. japonicus*. In contrast to literature data, hyperoside could not be detected in *L. cardiaca* but in both *L. japonicus* and *L. leonurus*. An instrumental HPTLC method for stachydrine was newly developed, using postchromatographic derivatization by Vágújfalvi reagent, an automatic TLC setup, and winCATS data analysis software. The results of an equally novel ^{1}H-qNMR procedure using its N-CH_3 singlet δ 3.03 ppm in comparison with the singlet of the two vinylic protons of the internal standard maleic acid at δ 6.18 ppm were always within the standard deviation of the HPTLC data, thus stachydrine could be quantified in every examined sample.

SL56 Aristolochia species: Toxicological risk assessment using a metabolomic approach

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Aristolochia species are toxic plants used as herbal medicines worldwide [1]. Their use has become a global public health concern [2] since they are known to cause aristolochic acid nephropathy, a devastating disease associated with kidney failure and kidney cancer [3]. The aims of this project are to assess the health risks associated with the use of different *Aristolochia* species and to elucidate the active principle behind their nephrotoxic effects. 44 medicinally used *Aristolochia* spp. were extracted with 70% methanol. The extracts were analyzed using LC-MS and NMR fingerprinting. The fingerprints were compared using principal component analysis and several aristolochic acid analogues were identified and quantified. The cytotoxicity and genotoxicity of 30 *Aristolochia* extracts was measured in human kidney (HK-2) cells. IC_{50} values were determined using the sulforhodamine B (SRB) assay [4]. Genotoxicity, apoptosis, cytotoxicity and cell cycle effects were measured using the flow cytometry-based micronucleus test (MNT). The content and nature of aristolochic acid analogues (AAAs) in *Aristolochia* species varies greatly. AA I and AA II are the most common AAAs, but aristolactam I, AA IV, AA C and AA D are widespread as well. In general, *Aristolochia* leaves contain less AAAs than seeds, roots and flowers. Extracts containing high amounts of AA I showed only moderate genotoxicity and cytotoxicity in HK-2 cells. On the other hand, micronuclei formation was also observed in extracts, which only contain aristolactams (e. g. *A. guentheri*). Therefore aristolactam I and related compounds need to be considered as potential nephrotoxic agents in *Aristolochia* spp. The pharmacological effects of these agents require further research. **References:** [1] Heinrich, M., et al (2009) J Ethnopharmacol, 125, 108 – 144. [2] Chen, C. H., et al. (2012) Proc Natl Acad Sci U S A, 109, 8241 – 8246. [3] Nortier J.L., et al. (2000) N Engl J Med, 342, 1686 – 1692. [4] P. Houghton, et al. (2007) Methods, 42, 377 – 387.

SL57 The estimation of factors defining the trace element structure of plants

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Nowadays, the microelement disbalanses are one of the prevalent diseases and the creation of effective herbal medicines for preventive maintenance and treatment of similar pathologies needs using the plants having an appropriate trace element structure. Therefore, the trace element structure must change in a defined range to make similar medicines with needed quality. In this connection the evaluation of factors which define trace element structure is the most important aim of the research. In family Boraginaceae there are a lot plants which can be used for making similar medicines and these plants were chosen as research objects. The concentration of 60 macro- and microelements in herbs of 55 species, 34 genera, 13 tribes and 3 subfamilies of family Boraginaceae from different habitats have been researched. The investigated plants were collected on Eurasia continent and Africa which are characterized by the most various conditions of growing. The amount of trace elements was determined by means of mass spectroscopy with inductively coupled plasma. The analysis of received experimental data was done by earlier created way based on using a multiple-factor analysis such as the cluster analysis. As a result the hierarchical tree was built and the good correlation between the place of plant in built hierarchical tree and its taxonomical position was shown. The biggest influence on trace element structure of plants renders evolutionary age and phylogenetic connections. Thus we speak about the existence of genetic determined element homeostasis in plants. Summarising the given results, it is possible to draw the following conclusions:

1. The element status of a plant is defined by its genome and correlates with the taxonomical position of plant well.
2. The element structure depends on growth conditions more than on element structure of soils
3. The element status of a plant has homeostasis property that allows to use plants for prevention of element disbalances.

SL58 Quality control of *Lycium barbarum* fruits: a valuable source of carotenoids

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Lycium barbarum L. fruits (Solanaceae), commonly known as Goji berries, are a traditional food and medicine in East Asia which nowdays is becoming widely popular in Europe and North America [1]. In view of its popularity robust quality control studies are necessary to assure its safety. In the framework of the preparation of TCM herbal drug monographs suitable for the European Pharmacopoea the analytical method for the quality of Goji berries was undertaken. A method based on HPLC-UV-DAD coupled to an ESI-MS interface was developed for the determination of the carotenoids in *L. barbarum* fruits. The method was simple and effective, taking into consideration most of the problematics regarding carotenoid analysis and is suitable for the routine control of *L. barbarum*. The method was further validated in terms of linearity and intermediate precision (different days and at three different concentration levels). All validation criteria were fulfilled. A reversed phase column was applied with isocratic eluent system consisting of acetone and methanol. Detection of carotenoids was at 450 nm. Carotenoids were expressed as beta carotene. Furthermore, an extraction protocol was developed and optimized in order to have a more accurate determination of the carotenoid content in *L. barbarum* fruits which is considerably higher than the one reported in the literature [2, 3]. Zeaxanthine palmitate was the main constituent in all tested commercial samples, varying from 56 – 84 mg 10 g^{-1} of edible portion. **References:** [1] Potterat O. Planta Med 2010; 76: 7 – 19. [2] Inbaraj BS, Lu H, Hung CF, Wu WB, Lin, CL, & Chen, BH. (2008), J. Pharm. Biomed. Anal, 47, 812 – 818. [3] Peng Y, Ma C, Li Y, Leung KS, Jiang ZH, Zhao Z. (2006). Plant Foods for Human Nutrition, 60, 161 – 164.

SL59 Safety data for herbal medicinal products: Assessment of genotoxicity

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The safety of herbal medicnal products (HMP) is usually supposed to be high, as it is documented by their long-standing therapeutic use. This view is generally accepted as far as it is based on endpoints which can be causally assigned to the product during clinical use. In the case of genotoxic effects this is not the case, as the manifestation of their consequences, as e. g. an enhanced incidence of cancer, would occur too late to allow an assessment of causality. Therefore, within the EU, an assessment of genotoxicity is a precondition for registration or approval of a

HMP. The committee responsible for HMPs at the European regulatory agency EMA, HMPC, has therefore published guidelines [1,2] which support the assessment of the whole range of preparations available from a herbal drug by means of a bracketing and matrixing approach. With the aim of providing such data for the most important herbal drugs used in Europe, Kooperation Phytopharmaka, a scientific society in the field of HMPs, is conducting a cooperation project [3]. Up to now, data on more than 30 herbal drugs have been generated, including artichoce, birch, ginseng, hawthorn, hops, horse chestnut, milk thistle, passion flower, sage, stinging nettle, St Johns wort, thyme and valerian. The project has addressed the question whether these herbs could be genotoxic and has generated data needed for regulatory submissions to drug regulatory agencies within the EU. **References:** [1] Guideline EMEA/HMPC/107079/2007. [2] Guideline EMEA/HMPC/67644/2009. [3] Kelber O., Steinhoff B., Kraft K., 2012. Assessment of genotoxicity of herbal medicinal products: A co-ordinated approach. Phytomedicine 19, 472, free access via http://www.koop-phyto.org

SL60 Beyond the African Herbal Pharmacopoeia: Recent progress towards new quality standards for African Herbal Products

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The African Herbal Pharmacopoeia (AfrHP) published in 2010 consisted of quality control standards for 52 of the most important African plants used in both traditional and modern medicine (see www.aamps.org). One of the aims of this publication was to establish trading standards for these herbal medicines in order to promote the use and trade in African Herbal Medicines. The publication received very positive reviews from several continents. One of the aims of the AfrHP published by the Association of African Medicinal Plants Standards (AAMPS) was to develop a living database to constantly update new information on the selected species. Subsequent to the publication there has been growing interest in the preparation of similar high quality monographs about African herbal plants used not just as human medicine but in cosmetics, perfumery and veterinary care. In South Africa a project has been started to develop a South African Indigenous Knowledge Pharmacopoeia. Meetings have recently been held in Kenya and South Africa to discuss possible extension of the AfrHP to include plants used for animal health and productivity. This paper is a progress report summarising recent developments including Africa wide research of AAMPS associates to prepare additional monographs for the African Herbal Pharmacopoeia. These include such interesting plants such as *Biophytum petersianum Klotsch, Galenica africana L., Ziziphus mauritania* Lam., *Acanthosicyos horridus* Welw. Ex Hook.f. (! Nara), *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Tsama Melon), *Colophospermum mopane* (J. Kirk ex Benth.) J. Kirk ex J. Léonard, *Schinziophyton rautanenii* (Manketti), *Sclerocarya birrea* (A. Rich.) Hochst. subsp. caffra (Sond.) Kokwaro (Marula), *Tarchonanthus camphoratus L.* (Camphorbush), *Ximenia americana L. Ximenia caffra* Sond. (Sour Plum), *Tribulus terrestris L.* Accurate monographs are required for Africa's rich biodiversity use human and animal health and welfare to attain its full potential.

Herbal medicinal products in animal healthcare and veterinary medicine

SL61 Research on herbal products for production of animals in the Netherlands

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Since 2006 RIKILT is involved in projects dedicated to herbal products for production animals. The FYTO-V project was aimed to increase the rational use of herbal therapies for prevention and treatment of diseases in organic husbandry. Therefore state-of-the-art herbal products were evaluated. On the Dutch Market 60 suppliers were identified, trading over 255 preparations for production animals. Herbal products were mostly marketed as either aromatic feed additives or complementary feeds. Very few were registered as veterinary medicines. Quality and safety of selected products were assessed by *in vitro* research, consisting of bioassays on anti-oxidants, antimicrobial activity and hormonal activity. The effects of selected herbal products were demonstrated through animal studies. The legislation of herbal products in Europe and other countries was also studied. Bottlenecks are less related to the substances than to the claimed efficacy. To improve scientific acceptation of herbal products a website (www.fyto-V.nl) was created with databases on herbs, activities, indications, animal species and literature. Also a course in phytotherapy for the higher levels of agricultural education was developed. In the Naturally Healthy project booklets were made for organic farmers (poultry, dairy and pigs) with information on the use of herbal and other natural products, general management tips and information on herbal products, literature and experiences in practice. English translations of the booklets are available via internet.

Tab. 1: Research on herbs for animals in the Netherlands

Project	Results
Fyto-V	Website: http: www.fyto-v.nl/en/index2.php
	Databases on the internet with herbs, activities, products and suppliers
	Course in phytotherapy for the higher levels of agricultural education
	List of 60 suppliers with 255 products for pigs, cows, and poultry
	Bioassays for effects on anti-oxidants, antimicrobial action,
	Immune modulating and hormonal action, identification of biomarkers for gut health
	Report on legislation of herbs in Europe with recommendations
Naturally Healthy	Practical guides for farmers: Natural Dairy Cow Health, Swine Health and Poultry Health.
Quality and safety	Overview of herbs used in production animals and horses
	Contaminants: heavy metals, mycotoxins, illegal additions, plant toxins in 53 products
	Research on: Hormonal action (oestrogenic and androgenic
	Batch to batch analysis
	Antimicrobial action
	Immune modulating action

The current project for the Dutch Government is on Quality and safety of herbal products for production animals. Here the use of herbal products in production animals was investigated and samples were analysed for plant toxins, contaminants, hormones and animal drugs. Moreover *in vitro* antimicrobial activity of 23 products was investigated and immunomodulatory action of herbs was reviewed. For an overview of the project results see table 1.

SL63 Veterinary use of *Cassia nigricans* Vahl: Donkey wound healing property

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Introduction: Ethnobotanical studies conducted in different areas in Mali (West Africa) demonstrated that *Cassia nigricans* is a traditional wound healing remedy (Diallo 2000). Cissé in 2009 showed that the

plant was the most reported one to treat animal wounds by breeders. **Objective**: The aim of this study was to verify the wound healing property of *C. nigricans* on wounded donkeys. **Methodology**: Donkeys naturally wounded during work were recruited by SPANA Mali (a donkey protection NGO) and classified in two groups of eight donkeys according to the location and size of their wounds. Group I was treated with *C. nigricans* and Group II with a control conventional wound spray. The plant material was composed of the areal part of *C. nigricans* which was dried and pulverized. A decoction was prepared with the powder of *C. nigricans* to clean wounds and the plant powder was applied onto the wound. The control used was a wound spray composed of gentian and oxytetracyclin. Dayly treatments were applied on a clean dried and measured wound. The size, the wound aspect and the treatment duration (number of day) were monitored during the healing process. **Results and discussion:** The mean wound contraction speed in *C. nigricans* treated group was 1.66 cm^2/day while 1.11 cm^2/day for the control group. The mean wounds size were 38 cm^2 in *C. nigricans* group and 45 cm^2 in the control group. The mean treatment duration was 27 days for *C. nigricans* and 37 days for the control, the difference was not significative according to the student test. The results showed a good wound healing property for *C. nigicans*. Polysaccharides are reported to be responsible for this property. Diallo in 2000 showed that *C. nigricans* water 100 °C extract contained 79% of carbohydrates. **References**: [1] Diallo D. 2000. Doctorate Thesis University of Lausanne [2] Cissé N.S. 200 Master thesis IPR/IFRA University of Bamako

SL64 Effects of saponin fractions from fenugreek and the soap bark tree in the diet on performance of Nile tilapia, *Oreochromis niloticus* (L.)

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Saponins are generally regarded as anti-nutritional factors in aquaculture diets. However, previous experiments have shown that low dietary levels of saponins derived from *Quillaja saponaria* Molina do have growth promoting effects on common carp and Nile tilapia. Based on these experiments, we conducted an experiment in which we fed eluated saponin fractions from *Q. saponaria* and *Trigonella foenum-graecum* L. (fenugreek) to Nile tilapia in a respirometric system allowing for continuous measurement of oxygen consumption. Saponins were eluated with consecutive methanol/water concentrations (v/v, 40/60, 60/40, 80/20) resulting in three different eluates for each plant. Fractions chosen were the 80% methanol eluate from *Q. saponaria* (80QS) and all three eluates from *T. foenum-graecum* (40TS, 60TS and 80TS). Three fish each were fed with low levels (150 mg kg^{-1} diet) of saponins in the diet and a control diet without saponins. Growth, feed and nutrient utilization, proximate composition, oxygen consumption and metabolic performance were evaluated. The fish grew between 224% (40TS) and 266% (Control) over the eight week period. Feed conversion ratios were between 0.94 (80TS) and 1.15 (40TS) and protein efficiency ratios between 2.54 (80TS) and 2.16 (40TS). Due to low sample sizes, no statistical differences were found between control fish and saponin fed fish. However, numerically one of the tested saponin fractions (40TS) showed inferior performance (Table 1). It is concluded that the tested saponins in the tested concentrations are no potential growth promoter for Nile tilapia. On the contrary, one fraction appears to be a growth inhibitor.

Tab. 1: Growth performance, feed conversion and nutrient utilization of the tilapia.

	Control	40TS	60TS	SOTS	SOQS
Initial body mass (g)	31.3 ± 8.25	39.0 ± 3.86	33.4 ± 8.21	35.8 ± 8.37	35.1 ± 2.52
Final body mass (g)	81.9 ± 14.5	86.3 ± 9.88	82.0 ± 5.13	92.5 ± 182	89.6 ± 14.0
Body mass gain (g)	50.7 ± 6.29	47.3 ± 11.9	48.6 ± 4.11	56.8 ± 102	54.5 ± 12.3
Growth (%)	266 ± 21.6	224 ± 41.4	253 ± 43.4	260 ± 15.0	255 ± 30.6
SGR (% day^{-1})	1.74 ± 0.15	1.42 ± 0.33	1.64 ± 0.32	1.71 ± 0.10	1.66 ± 022
MGR (g $kg^{-0.8}$ day^{-1})	7.01 ± 0.34	5.82 ± 1.44	6.60 ± 1.21	7.05 ± 0.36	6.85 ± 1.06
Feed conversion ratio	0.96 ± 0.04	1.15 ± 0.30	1.03 ± 0.20	0.94 ± 0.05	0.98 ± 0.15
Protein efficiency ratio	2.49 ± 0.11	2.16 ± 0.55	2.36 ± 0.42	2.54 ± 0.13	2.47 ± 037
Protein productive value (%)	41.4 ± 4.40	36.8 ± 9.40	38.0 ± 4.14	42.1 ± 0.61	413 ± 3.92
Apparent lipid convers. (%)	62.1 ± 4.56	56.2 ± 25.0	52.2 ± 172	672 ± 7.44	61.6 = 14.6
Feed intake (g DM)	48.7 ± 8.23	52.2 ± 2.71	49.7 ± 5.80	53.3 ± 9.02	52.3 ± 5.05

Values are expressed as mean ± SD: n = 3, TS = *Trigonella foenum-graecum* saponins, QS = *Quillaja saponaria* saponins, SGR = specific growth rate, MGR = metabolic growth rate

SL65 Extracts of ethnoveterinary plants used to control myasis caused by blowflies in animals are effective in several mechanisms and may have valuable practical applications

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Myiasis caused by blowfly larvae is a serious problem affecting animal health and commercial production especially of sheep all over the world. The flies have developed resistance against the commercial pesticides used. We investigated seven plant species used by rural pastoralists to treat these infestations. Extracts of several plant species had good antimicrobial activity (MICs 0.04 to 0.6 mg/ml) against pathogens infecting wounds. Because flies are attracted by volatiles emitted by these microorganisms, treating wounds with such an extract would limit infestation. The activity of extracts on killing, paralysing and metamorphosis of third instar larvae of *Lucilia cuprina* and *Chrysomya marginalis* (Diptera: Calliphoridae) were investigated. Several species had promising activities on the larvae. Dose related response activities were determined on four of the most promising plant species (*Aloe zebrina* Baker, *Clausena anisata* (Wild) Hook. f. ex. Benth, *Erythrina lysistemon* Hutchand, *Spirostachys africana* Sond.). The parameters investigated were larval behaviour, larval development, larval emergence of adult flies, decreased ingestion of meat by the larvae, pupae mass and adult emergence rates. For *C. anisata* and *S. africana* extracts the increase in the concentration was also associated with larvae circling on top of the plastic cups possibly indicating repellency and the emerging adult flies being smaller. *C. anisata* was selected for field evaluation of blow flies on two farms. Larvae exposed to liver baits treated with *C. anisata* developed slower, had a prolonged larval period, smaller body size, sluggish behaviour, delayed pupation and reduced eclosion rates in comparison to the controls. The active compounds from *C. anisata* were isolated and characterized. The results have been patented. **References:** [1] Mukandiwa L, Eloff JN, Naidoo V (2012) Veterinary Parasitology 190, 566 – 572. [2] Mukandiwa L, Eloff JN, Naidoo V (2012) Journal of Ethnopharmacology 143, 812 – 818

SL66 New forms of vitamin D for animal and human health from plant origin

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In modern animal meat production, undersupply of vitamin D results in skeletal problems because, and especially in poultry rearing, bone growth does not keep up with overall growth. However, Vitamin D itself does not possess biological activity but is transformed in 2 hydroxylation steps in the liver and kidney, respectively, into the active form 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) which activates a gene inducing the synthesis of calbindin, the calcium binding protein responsible for the uptake of calcium from nutrition. Without the active metabolite $1,25(OH)_2D_3$ no calcium can be absorbed, even if there is enough calcium contained in the diet. Direct application of $1,25(OH)_2D_3$ has been found to be the most active agent to prevent leg weaknesses and lameness in growing broiler chickens, in particular in modern breeds. A report of the scientific committee of the European Commission confirmed $1,25(OH)_2D_3$ as the most effective agent in the prevention of tibial dyschondroplasia. Because synthetic $1,25(OH)_2D_3$ is expensive and not

available for animal nutrition, an alternative has been found in the plant *Solanum glaucophyllum* which contains the active form of Vitamin D_3 in glycosidically bound form. In order to exploit this plant as a source of active vitamin D, a unique standardized and formulated herbal product for animal nutrition containing $1,25(OH)_2D_3$-glycosides as active components was developed. This affords better pharmacokinetic properties and turns the product into a slow release form with little danger of overdosing.

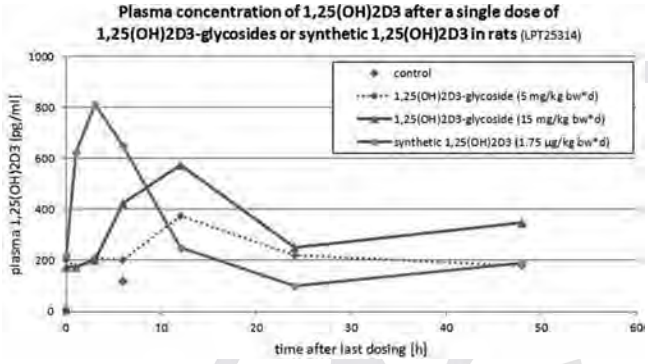

Fig. 1: Difference in plasma concentrations of 1,25-dihydroxyvitamin D_3 after a single dose of free 1,25-dihydroxyvitamin D_3 and a herbal extract of *Solanum glaucophyllum* after a single application in rats.

Reference: [1] EUROPEAN COMMISSION, the Welfare of Chickens Kept for Meat Production (Broilers), Report of the Scientifc Committee on Animal Health and Animal Welfare, page 33, adopted 21 March 2000, SANCO.B.3/AH/R15/2000

SL67 Sainfoin – new data on anthelmintic effects and production in sheep and goats

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Gastrointestinal nematodes (GIN) are one of the most important problems affecting health and therefore performance and welfare in small ruminant husbandry. The control of these parasites in the past strongly relied on the repeated use of anthelmintic drugs. This has led to nematode populations which are resistant to most of the currently available anthelmintics. Furthermore customer's demands for organic and residue free animal products are increasing. The aforementioned problems have given a strong impetus for the development of new non-chemical strategies to control GIN. Previous research has pointed out the anthelmintic potential of sainfoin (*Onobrychis viciifolia*, cv. Visnovsky) and other tanniferous (CT) feed sources in goats and lambs infected with GIN. A recent Swiss experiment focussed on the use of sainfoin and field bean (*Vicia faba*, cv. Scirocco) as single CT sources as well as in combination for additional synergic effects, to reduce periparturient GIN egg rise of ewes in late gestation and early lactation. Another experiment with Alpine goats concentrated on the influence of sainfoin on milk performance and cheese quality. The results of these experiments will be presented and discussed in connection with previous knowledge on (i) anthelmintic effects of sainfoin and (ii) the influence of sainfoin administration on performance.

Miscellaneous

SL68 Effective plant reproduction of *Pelargonium sidoides* by using somatic embryogenesis

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Roots of the native South African medicinal plant *Pelargonium sidoides* DC are used for the production of the herbal medicinal product Umckaloabo® which is approved for the treatment of acute bronchitis. Initially, the plant material only originated from wild collections. Since some years the root material increasingly is derived from plant agriculture of *P. sidoides*. Nevertheless the population of wild growing plants during the last years decreased by half (1). With regard to species protection in combination with an increasing demand of plant material for the industrial production of the medicinal product, the requirement of an effective method for the propagation of *P. sidoides* becomes obvious. With somatic embryogenesis, a cell culture technique for plant reproduction, embryos could be generated from somatic cells of blossom stems of *Pelargonium*. A one-week cultivation of the plant explants in media containing specific phytohormones followed by a cultivation period without phytohormones resulted in the induction of numerous somatic embryos within 3 – 4 weeks. A treatment of explants with a specific purified extract leads to improved somatic embryogenesis. The method allows an enhanced production of numerous clones from one plant (e.g. interesting chemical races) and represents an effective way of plant reproduction. **Reference:** [1] Newton, D. (2008): Development of a non-detriment finding process for *Pelargonium sidoides* in Lesotho. International Expert Workshop on CITES Non-Detriment Findings. Cancun, Mexiko, 2008.

SL69 Anti-viral and anti-inflammatory efficacies of Sinupret® dry extract BNO 1011 rationalise its therapeutic use in acute rhinosinusitis

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The herbal medicinal product Sinupret® (Gentianae radix, Primulae flos, Sambuci flos, Rumicis herba, Verbenae herba) is used for the treatment of acute rhinosinusitis. Its pathophysiology is dominated by inflammation in nasal/paranasal mucosae, mainly triggered by infection with respiratory viruses like human influenza A. We investigated the antiviral and anti-inflammatory activity of Sinupret® dry extract BNO 1011 *in vitro* and *in vivo*. BNO 1011 was tested against two human influenza A H1N1 (Flu A) strains with divergent sensitivity against the neuraminidase inhibitor oseltamivir (OS) in a plaque reduction test and for neuraminidase inhibition of the same Flu A strains. The anti-oxidative capacity of BNO 1011 was evaluated by monitoring the diphenylpicrylhydrazyl (DPPH) radical scavenging activity. In the carrageenan-induced pleurisy model in rats, BNO 1011 (100 mg/kg and 500 mg/kg, p.o.) was analysed for suppression of pro-inflammatory parameters [exudate formation, neutrophil infiltration, prostaglandin E $(PGE)_2$ levels; pulmonary cyclooxygenase (COX)-2]. BNO 1011 blocked Flu A replication with an EC_{50} of 8 µg/mL for each strain tested. As underlying mechanism, neuraminidase inhibition was identified (IC_{50}: 59 µg/mL and 100 µg/mL, respectively), irrespective of the strains' sensitivity against oseltamivir. Regarding the anti-inflammatory activity, BNO 1011 reduced the DPPH radical concentration (IC_{50}: 46 µg/mL) *in vitro*. Moreover, orally applied BNO 1011 significantly reduced exudate volume, neutrophil influx (100 and 500 mg/kg), PGE_2 levels and COX-2 expression (500 mg/kg) in acute pleurisy. Together, BNO 1011 acted against the cause of respiratory viral infection and interfered with the inflammatory collateral damage. These findings support the application of BNO 1011 in the treatment of acute, viral rhinosinusitis.

SL70 Calcium antagonistic effects of ethanolic myrrh extract in inflamed intestinal smooth muscle preparations

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Myrrh is the oleo-gum resin of mainly *Commiphora molmol* ENGLER (Burseraceae) and as powdered substance one compound in the traditional medicinal product Myrrhinil-INTEST®, which has been used for the treatment of unspecific and inflammatory intestinal disorders. To date only limited data is available regarding its mechanism of action. Besides antimykotic and antiphlogistic properties calcium antagonistic and antidiarrhoic effects have been discussed [1]. The aim of the present study was to evaluate the calcium antagonistic effect of myrrh. Therefore, an ethanolic myrrh extract (MY) was tested for its effects on muscle tone and acetylcholine (ACh)-induced contractions in untreated and inflamed rat ileum/jejunum preparations. Inflammation was experimentally induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS, 10 mM, 30 min). Additionally, the effect of the calcium channel agonist Bay K8644 in presence of varying MY concentrations was examined to con-

firm the calcium antagonistic effect. MY suppressed the ACh-induced contraction down to 25,75% (0,99 mg/ml MY). MY (0,15; 0,25 and 0,35 mg/ml) induced a concentration-dependent right-ward shift of the Bay K8644 concentration-response curve in untreated and inflamed preparations with a significant EC50 shift. Schild analyses resulted in a pA2 value of 0,93 for untreated preparations. Increasing MY concentrations induced a concentration-dependent decrease of the agonistic maximum effect in untreated and inflamed preparations down to 15,84% and 25,78% respectively for the highest concentration leading to a pD2 value of 0,58. MY reduced intestinal muscle tone and ACh-induced contraction of untreated and inflamed ileum/jejunum preparations based on dual calcium antagonism characterised by a right-shift of the agonistic dose-response curve and a depression of maximum effect. The resulting reduction of intestinal motility and spasmolytic effects provide a rationale for the treatment of intestinal disorders.

SL71 Anti-cancer thymoquinone from *Nigella sativa*

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Quinones and phenolic compounds are important bioactive natural products in medicinal and dietary plants [1 – 3]. For example, thymoquinone (2-isopropyl-6-methyl-1,4-benzoquinone, TQ) is the main bioactive compound from *Nigella sativa* (black cumin) (Figure), showing various biological activities including anti-cancer activities [4]. Although much effort has been made to understand the mechanism of action of TQ, it is still elusive. TQ is not a fully substituted quinone which should be reactive towards thiol groups of proteins [5 – 7]. Here, we report the synthesis of deuterated TQ as a molecular probe for the protein targets in ovarian cancer cells and leukaemia cells and a stable internal standard for the accurate quantification of TQ (stable isotope dilution method) in plant materials using mass spectrometry. In addition, TQ has been shown to bind to cysteines of haemoglobin and glutathione by HPLC and LC-ESI-MS, which indicates a possible covalent modification of protein targets in cancer cells. A MS-based metabolic approach has also been used to understand the change of metabolites in K562 leukaemia cells after addition of TQ. The significant decrease of many intermediates of TCA cycle and lipids has been observed. Moreover, a number of thioether and amino TQ analogues are prepared for the study of structure-activity relationships, in particular, to prove if the thiol addition reaction is responsible for the biological activity of TQ and search for more potent analogues.

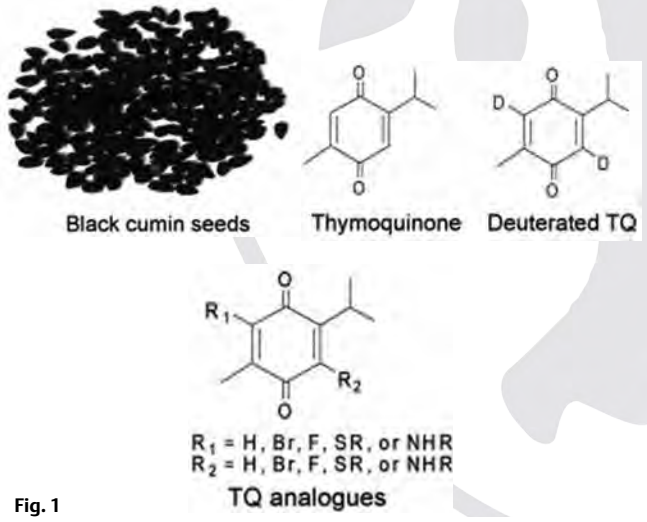

Fig. 1

References: [1] Link A, Balaguer F, Goel A. (2010) *BIOCHEM. PHARMACOL.*, **80**, 1771 – 1792. [2] Li WW, Barz W. (2005) *TETRAHEDRON LETT.*, **46**, 2973 – 2977. [3] Li WW, Barz W. (2006) *PLANTA MED.*, **72**, 248 – 254. [4] Woo C C, Kumar AP, Sethi G, Tan KH. (2012) *BIOCHEM. PHARMACOL.*, **83**, 443 – 451. [5] Li WW, Heinze J, Haehnel W. (2005) *J. AM. CHEM. SOC.*, **127**, 6140 – 6141. [6] Li WW, Hellwig P, Ritter M, Haehnel W. (2006) *CHEM. EUR. J.*, **12**, 7236 – 7245. [7] Lu S, Li WW, Rotem D, Mikhailova E, Bayley H. (2010) *NATURE CHEM.*, **2**, 821 – 828.

SL72 Characterisation and specificity of different spermidine synthases

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The polyamines spermidine, spermine and thermospermine are small molecules occurring in humans, in animals and plants, and also in bacteria and fungi. Under physiological conditions they are protonated cations allowing interactions with negatively charged macromolecules such as nucleic acids and membranes. Polyamines are involved in the cell stress response, in cell differentiation and growth processes. They also have regulatory and cell protective functions. Polyamine metabolism is a target for therapy. Tumour cells often show deviating polyamine patterns, and *Plasmodium* and other pathogenic protozoa depend on polyamine uptake from internal bacteriods (apicoplasts). The *in vivo* formation of the different polyamines is accomplished by several enzymes. Spermidine synthase (Fig. 1) is a key enzyme for higher polyamines transfering an aminopropyl group onto putrescine forming spermidine (Fig. 2). The cosubstrate dcSAM (decarboxylated *S*-adenosyl-L-methionine) serves as aminopropyl group donor.

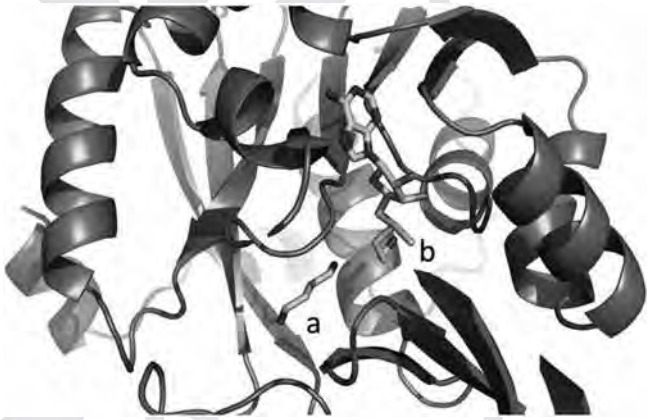

Fig. 1: Human spermidine synthase, section of the catalytic site is shown with a, putrescine and b, dcSAM created with Pymol 1.3, based on PDB 2O0L (human spermidine synthase).

In order to target spermidine synthases from particular organisms selectively, information on substrate and cosubstrate binding is necessary. Analogues of substrate and cosubstrate, partially obtained by synthesis, are applied to explore spermidine synthase binding specificity. Active site amino acids are exchanged by site-directed mutagenesis to learn about their significance in substrate and cosubstrate binding and on the catalytic mechanism.

SL73 *In vitro* transport studies with kaempferol and its anxiolytic metabolite 4-HPAA in human cell models

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Previous pharmacological investigations have shown that the flavonoid kaempferol induces anxiolytic activities in mice after oral administration, but not after intraperitoneal (i. p.) application. However, i. p. administration of 4-hydroxyphenylacetic acid (4-HPAA), a major metabolite of kaempferol formed by the intestinal microflora, induces behavioral

changes. Kaempferol was thus considered to be a prodrug. Orally administered CNS active compounds have to be absorbed in the intestine and reach the CNS as target tissue. With the aid of *in vitro* cell models, we investigated the ability of kaempferol and its main metabolite 4-HPAA to cross the intestinal and the blood brain barrier (BBB). Established BBB (human brain capillary endothelial cell line; hBMEC) and human intestinal transport (Caco-2 cells) models were selected for compound permeability prediction. As a first step, reliable and robust quantitative UPLC-MS/MS assays were developed and validated for determination of kaempferol and 4-HPAA in the transport media. Methods were validated according to FDA guidance over the range of 20 ng/ml (LLOQ) to 2000 ng/ml (ULOQ). Quantitation of kaempferol was carried out in positive electrospray ionization (ESI) mode with ^{13}C-labeled kaempferol as internal standard (IS). The method for quantification of 4-HPAA was developed in negative ESI mode and vanillic acid served as IS. The concentration/response data of both analytes fitted with a quadratic curve with $1/X^2$ weighing. The average coefficients of determinations (R^2) were greater than 0.995. The assays were used for transport studies with cell layers grown in transwell plates. The results from this study, combined with bioanalytical findings from *in vivo* studies with oral application of kaempferol and phenylacetic acids, will help to clarify the role of flavonoids and their metabolites in the behavioral effects observed *in vivo*.

SL74 Inhibition of aquaporin-1 but not aquaporin-4 water permeability by bacopaside I derived from *Bacopa monnieri*

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Aquaporin (AQP) water channels mediate transmembrane water permeability and maintain body fluid homeostasis. They are widely expressed in human body including proximal tubules in kidney (AQP1) and endfeet of astroglia cells in brain (AQP4). *Bacopa monnieri* has been used in traditional Indian medicine for centuries to improve memory. Recent study has demonstrated the antitumor effect of bacopa in several cancer cell lines in which AQP expression is upregulated [1]. We hypothesized the block of AQP water channel by bacopa and its derived molecules. Whole bacopa plant was dried and extracted in methanol reflux. We show that the osmotic water flux in AQP1-expressing *Xenopus lavis* oocytes was reduced by pre-incubation in saline containing bacopa methanol extract (BME). BME was further fractionated and the active compound has been identified as bacopaside I (BI) by both NMR and mass spectrography. BI inhibits AQP1 water permeability (IC_{50} ~ 118 mM, applied extracellularly) but not AQP4. The efficacy of block was significantly impaired by mutagenesis of intracellular AQP1 arginines-159, 160 to alanines (R 159,160 A), which supports the binding of BI to the intracellular loop D region. In silico docking of BI further supported the loop D binding and suggested the occlusion of AQP1 water pore by BI. Our study identified BI, a phytochemical isolated from medicinal plant *Bacopa monnieri*, is an AQP type-selective pharmacological agent. With further *in vivo* study and chemical modification, BI will be a breakthrough strategy in treatment of AQP1 related disorders.

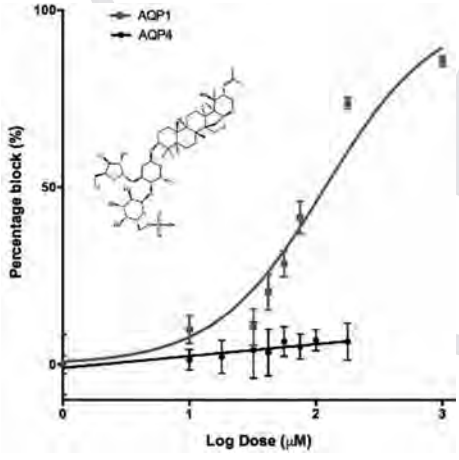

Fig. 1 An active component of *Bacopa monnieri*, Bacopaside I (insert) causes dose-dependent block of wild type AQP1 but not AQP4 channels.

References: [1] Peng L, Zhou Y, Kong de Y et al. Antitumor activities of dammarane triterpene saponins from Bacopa monniera. Phytotherapy research: PTR 2010; 24: 864 – 868

SL75 The biocatalytic capacity of tropinone reductase-like enzymes from plants

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Tropinone reductases (TRs) are short-chain dehydrogenases/reductases (SDRs) involved in tropane alkaloid biosynthesis. TRs reduce tropinone stereospecifically either to tropine (3a-tropanol), precursor of classical tropane alkaloids like hyoscyamine and scopolamine, or to pseudotropine (3b-tropanol), precursor of nortropane alkaloids e.g. calystegines. TRs were cloned and expressed from several tropane and nortropane alkaloid containing Solanaceae. In addition, one tropane alkaloid forming species of the Brassicaceae, *Cochlearia officinalis*, contains a special tropinone reductase (CoTR), which reduces tropinone to pseudotropine and tropine *in vitro*. Based on sequence similarity, a large number of SDR genes from diverse plants were annotated to code for putative TRs or TR-like enzymes (TRLs). Many of those plants do not contain tropane or nortropane alkaloids at all (e.g. *Arabidopsis thaliana*, Brassicaceae) or they accumulate calystegines only, e.g. Solanum species. The metabolic role is not known for any of those TRLs. SDRs accept a large variety of substrates, and one individual SDR often accepts several substrates. Accordingly, many tropinone reductase-like enzymes reduce various carbonyl compounds *in vitro*, among them terpene ketones, but no tropinone. Reduction was stereospecifically and the corresponding alcohol was oxidised stereoselectively. Thus, application of TRLs for directed biocatalytic conversions appears attractive. Carbonyl structures that were identified *in vitro* to serve as substrates for TRLs were used for pharmacophore searches *in silico*. A data base screening with those pharmacophores led to the identification of additional potential substrates that will be confirmed by turnover experiments *in vitro*.

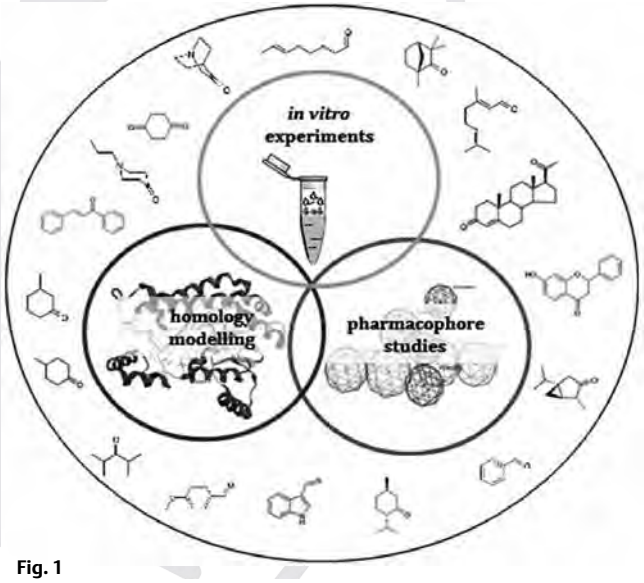

Fig. 1

SL76 Identification of a MYRII myrosinase gene in horseradish (*Armoracia rusticana*), expression in *Pichia pastoris* and characterization of the recombinant enzyme

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Horseradish (*Armoracia rusticana*) is a perennial crop plant belonging to Brassicaceae family. Its storage roots are used as a condiment because of the hot taste caused by isothiocyanates. These compounds with several

health-beneficial effects are released from glucosinolates by the enzyme myrosinase. Several myrosinase-like genes belonging to different subfamilies have been identified in the *Arabidopsis thaliana* genome. Horseradish possesses at least one myrosinase belonging to the MYRII subfamily. A full-length cDNA encoding a new myrosinase was isolated from horseradish. The gene (*ArMY2)* is specifically expressed in primary roots of young plants. *ArMY2* was over-expressed in *Pichia pastoris* and the recombinant enzyme was characterized biochemically. Substrate affinity was about 5 fold higher towards gluconasturtiin and towards sinigrin. Interestingly, gluconasturtiin was found to be the most abundant glucosinolate in primary horseradish roots. In contrast, sinigrin was dominating in storage roots and leaves.

SL77 Discovery of an alliinase association with a legumin-like storage protein in *Allium* species subgenus *Melanocrommyum*

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The genus *Allium* with more than 800 species is one of the largest genera in the plant kingdom [1]. Only a few species thereof are of great economic importance, especially *Allium cepa* and *Allium sativum* due to their usage as a condiment. However, in Central Asia other species, especially *A. stipitatum*, belonging to subgenus *Melanocrommyum*, are consumed frequently. All *Allium* species express an alliinase which cleaves cysteine sulfoxides after cell disruption leading to the formation of aroma compounds [2]. Only few other proteins contained in *Allium* were investigated. In *A. sativum*, a mannose-binding lectin was discovered, happening to be associated with alliinase [3]. Besides lectin, no further storage proteins in *Allium* have been reported yet. On an SDS-gel using reducing conditions, two intense protein spots can be seen between 20 and 30 kDa being characteristic in protein extracts of *Melanocrommyum* species. Alliinase is always associated with them. Further investigation revealed that those protein subunits are parts of a single protein connected to each other by disulfide bonds. N-terminal sequencing indicated a conserved domain of legumin-like storage proteins. Sequencing with mass spectrometry after trypsine digestion allowed the creation of primers and to obtain the middle part of the gene of that storage protein by PCR. The sequence was amplified to the flanking ends using the method of restriction enzyme site-directed amplification [4]. Thus knowledge of the DNA sequence of that storage protein was achieved. The resulting amino acid sequence has almost no analogy to known sequences. In the ongoing research, the question of modification of the alliinase function by this protein has to be answered. **References:** [1] Fritsch RM et al. Phyton (Horn, Austria) (2010) 49(2):145 – 220 [2] Stoll A, Seebeck E, Helvet Chim Acta. (1949) 32(1): 197 – 205 [3] Rabinkov A, Wilchek M, Mirelman D, Gycoconj j. (1995) 12(5):690 – 698 [4] Gonzáles-Ballester D et al. Anal Biochem. (2005) 340(2):330 – 5

SL78 Biochemical fingerprinting of endophytes harbored in *Radula marginata* that confer plant fitness benefits

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Our work focusses on the assessment and elucidation of the cost-benefit interactions of a special group of microorganisms known as endophytes which inhabit the internal tissues of the host plants without causing any immediate negative effect, and remain in a mutualistic association for at least a part of their life cycle. Plants are known to produce various bioactive secondary metabolites as defensive compounds. Cannabinoids are the most extensively studied secondary metabolites of *Cannabis sativa* L. plants. Recent work on liverworts like *Radula marginata* led to the identification of new cannabinoids with structural similarity to tetrahydrocannabinol, the major psychoactive compound of *Cannabis* plants. We have isolated a plethora of endophytes, both fungi and bacteria, from *R. marginata*. Since both *Radula* and *Cannabis* contain similar biosynthetic principles, we are evaluating the biocontrol potential of the endophytes against the host specific phytopathogens of *C. sativa* L. plants namely, *Botrytis cinerea* (causing gray mold disease) and *Trichothecium roseum* (causing pink rot disease), respectively. We are investigating the various attack-defense-counterdefense responses of the isolated endophytes when challenged by the phytopathogens. These responses trigger the production of secondary metabolites or intermediates which are otherwise 'cryptic'. We are not only analyzing the various cost-benefit tradeoffs between the endophytes and host plants but also evaluating the bioactive target and/or non-target metabolite production correlating to the endophyte-pathogen interactions. This will enable us in understanding the biochemical fingerprint of the endophytes which aid in thwarting the phytopathogens and reducing the loss of such therapeutically beneficial plants. **References:** [1] Kusari et al. (2012) Fungal Divers In Press (doi. 10.1007/s13225 – 012 – 0216 – 3) [2] Kusari et al. (2013) In: Kharwar R.N. (ed.) Endophytes, (ICPMB 2012), Springer-Verlag, Heidelberg. In Press

SL79 Introduction of qNMR to the Japanese Pharmacopoeia (JP) for specification of marker compounds used for standardization of herbal medicines

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In Japan, standardization of herbal medicines is mostly controlled by the Japanese Pharmacopoeia (JP). JP16 contains the monographs of 217 crude drugs inculding powders, 22 Kampo extracts and 32 crude drug preparations other than Kampo extracts. The specific marker compound for quantification is very important for standardization of herbal medicines. Therefore, JP has prepared several marker compounds as JP Certified Reference Standards (JP-CRS), which are highly purified and of which the water contents are known. But, it is difficult to prepare them because of the following reason. The synthesis of natural compound is not so easy in most cases. Therefore the targeted compound is separated from natural materials with a great deal of effort requiring high economical cost. 2) Karl Fischer method is necessary to determine water contents precisely, and consequently the valuable separated compounds are consumed for the determination of water content and this also leads to high economical cost. Considering these difficulties, JP utilizes many chemical reagents commercially available as reference standards for quantitative analyses instead of JP-CRS. However, there is no information on their absolute purity. In order to solve the issues, in 2009 the JP experimental group started the joint research [1] for utilizing quantitative NMR (qNMR) to determine the absolute purity of chemical reagents used for assay of herbal medicines. As a result, in 2013, four reference standards (geniposide, magnolol, paeonol and magnoflorine) having absolute purity values determined by qNMR in a reagent company are available in the markets and the quantitative HPLC assays by using these reference standards will appear in several monographs in JP16 supplement 2. **References:** [1] Hosoe J. et al., Pharmaceutical and Medical Device Regulatory Science, **41**, 960 – 970 (2010); [2] Hosoe J. et al., ibid., **43**, 182 – 193 (2012); JP16 Supplement 1: http://www.mhlw.go.jp/topics/bukyoku/iyaku/yakkyoku/english.html

Poster presentations

A. Natural products against neglected diseases

PA1 Role of *Helicobacter pylori* infection in gastric carcinogenesis & probiotic treatment *in vitro* and in an animal model

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Tumors of stomach, liver, and lower bowel are the second, third, and fourth leading causes of human cancer mortality, together accounting for more than two million deaths annually. Michetti et al.,(1999); Shibuya et al., (2000) reported that ~10% of the world's total cancer burden and 20 – 30% of deaths are attributable to infections of the gastrointestinal (GI). The aim of this study was to present an animal model for studying the pathology and mechanism underlying *Helicobacter pylori*-induced gastric cancer. To prove that probiotic micro-organisms may be used as a possible tool for the management of *H. pylori* infection in vitro Several nested case-control studies have reported the potentially causal relationship between *Helicobacter pylori* infection and the development of gastric cancer. We presented an animal model for studying the pathology; mechanism underlying *H. pylori*-induced gastric cancer and the role of *lactobacillus species* use as a probiotic treatment. Furthermore, the carcinogen A'-methyl-A'-nitrosourea (MNU) was used to in-

crease the gastric cancer incidence in *H. pylori* infected animal model.- The present findings demonstrated that *H. pylori* infection increased the incidence of MNU-induced adenocarcinoma of the glandular stomach in mice i.e. *H. pylori* can be considered a promoter for a carcinogen action In addition, *Lactobacillus* was effective as probiotic by a percentage of 59% in vitro. Our results confirmed the antagonistic effect of *lactobacillus on H. pylori* In accordance to Sgouras et al., (2005); Lesbros-Pantoflickova et al., (2007) findings, who stated that animal studies demonstrated that probiotic treatment is effective in reducing *H. pylori*-associated gastric inflammation.

PA2 The alkaloid fraction from *Buxus sempervirens* leaves shows strong in vitro activity against *Plasmodium falciparum*

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In ethnopharmacology, *Buxus sempervirens* (European Box, Buxaceae) is known as a plant with antimalarial activity [1,2]. On different continents, species of the genus *Buxus* were used in different preparations to cure malaria [3]. In the course of our ongoing screening of plant extracts for antiprotozoal activity, a CH_2Cl_2 extract from the leaves of *B. sempervirens* showed selective *in vitro* activity against the NF54 strain of *Plasmodium falciparum* (IC_{50} = 2.79 and 20.2 µg/mL for antiplasmodial and cytotoxic activity, respectively). Separation of the extract by acid/base extraction into a basic and a neutral non-polar fraction (extraction with acetic acid/water followed by neutralization and extraction with CH_2Cl_2) led to a much more active and equally selective fraction with alkaloids (IC_{50}= 0.36 and 7.3 µg/mL, respectively) while the fraction of non-polar neutral constituents was markedly less active than the crude extract (Table 1). Thus, the activity of the crude extract can clearly be attributed to alkaloid constituents. Identification of the main triterpene-alkaloids and characterization of the complex pattern of this alkaloid fraction was performed by UHPLC/+ESI-QTOF MS analyses.

Tab. 1: *In vitro* activity of CH_2Cl_2 extract of *B. sempervirens* leaves and its main fractions against *P. falciparum* and cytotoxicity data against L6 rat skeletal myoblasts. Data represent IC_{50} values in µg/mL. Chloroquine and Podophyllotoxin are positive controls.

	P. falciparum NF54 strain	L6 cells
CH_2Cl_2 extract	2.79	20.2
Alkaloid fraction	0.361	7.31
Lipophilic fraction	7.76	33.9
Chloroquine (pos. control)	0.003	-
Podophyllotoxin (pos. control)	-	0.008

ESI-MS/MS target-guided larger scale preparative separation of the alkaloid fraction (4 g) was performed by '*high-speed-* ' and '*spiral coil*-countercurrent chromatography'. Subfractions containing all representative alkaloids are currently under evaluation for their *in vitro* antiplasmodial activity. The results of these tests will enable us to identify and isolate the most active alkaloids for further testing and evaluation. **References:**
[1] Leporatti ML et al., Journal of Ethnopharmacology, 14, 53 – 63, (1985). [2] Orhana IE et al., Industrial Crops and Products, 40, 116 – 121, (2012). [3] Athar A in: The Alkaloids, Cordell AC (Ed.), Vol. 66, pp.191, Elsevier, 2008 This work is part of the activities of ResNetNPND: http://www.uni-muenster.de/ResNetNPND/

PA3 Bioassay-guided isolation of alkamides from an extract of *Achillea ptarmica* L. with antiprotozoal activity

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In the course of an ongoing screening of the family Asteraceae for antiprotozoal activity [1], a CH_2Cl_2- extract from the flowering aerial parts of *Achillea ptarmica L.* (sneezewort yarrow) was active in vitro against *Trypanosoma brucei rhodesiense* (IC_{50}= 0.67 µg/mL) and *Plasmodium falciparum* (IC_{50}= 6.58 µg/mL).

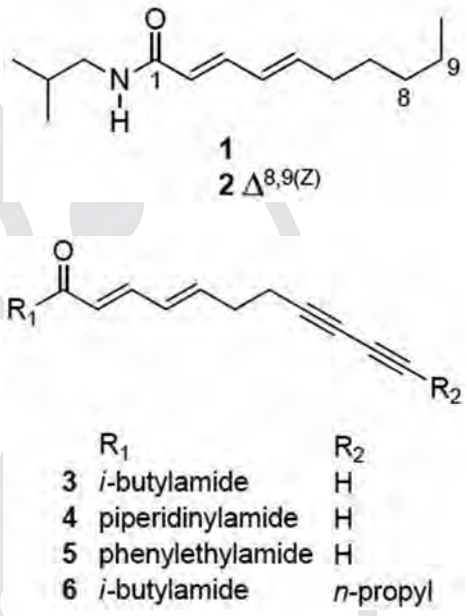

Fig. 1

A bioassay guided fractionation by CC on silica followed by preparative HPLC led to the isolation and identification of five alkamides from the most active fractions. Pellitorine (1) and 8,9 *Z*-dehyropellitorine (2) are the main components of the extract. Beside these olefinic acid amides, four alkamides with diene-diyne structures (3 – 6) were isolated. Of these, 4 and 5 represented a mixture (2:1). Compounds 1-6 were tested for antiprotozoal activity in vitro (Table 1).

Tab. 1: In vitro antiprotozoal and cytotoxic activity of alkamides isolated from *A. ptarmica*. Data represent IC_{50} values in µg/mL

Compound	*T.b. rhodesiense* (STIB 900)	*P. falciparum* (NF54)	L6 cells
1 (pellitorine)	5.35	3.25	45.0
2 (8,9 Z-dehydropellitorine)	2.00	6,47	16.5
	6.66		
4+5 (2:1)	3.50	6,89	43.4
6 (anacycline)	5.12	under evaluation	47.6
Melarsoprol (pos. control)	0.002	–	-
Chloroquine (pos. control)	–	0.003	
Podophyllotoxin (pos. control)	–		0.008

Pellitorine is the most active compound so far within this study against *P. falciparum*, while the 8,9-dehydro-derivative (2) is the most active compound against *T. b. rhodesiense.* None of these alkamides, however, was as active against *T. b. rhodesiense* as the crude extract so that more active constituents still remain to be identified. **References:**
[1] Gökbulut A, et al. Planta Med, 78, 225 – 229 (2012). This work is part of the activities of ResNetNPND: http://www.uni-muenster.de/ResNetNPND/

PA4 Antibacterial constituents from the roots of *Caryopteris mongolica* Bge.

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In the course of our study on *in vitro* screening of Mongolian plants, the roots of *Caryopteris mongolica* have showed strong activity against Gram-positive bacteria. A literature search revealed also that a few reports on chemical composition of this plant [1,2]. Therefore, we have continued to isolate antibacterial constituents from the roost of *C. mongolica*. *C. mongolica* Bge. (Verbenaceae) has long been used as a traditional medicine to stop bleeding after giving a birth, to contract uterus, and to treat a fever in Mongolia [3]. Chloroform extract of the roots of *C. mongolica* was passed through a column of silica gel. The bioactive fraction was further purified using both an ODS column and HPLC, led to the isolation of the three new compounds (**1** – **3**, Figure) and five known abietane diterpene derivatives, namely, demethylcryptojaponol (**4**), incanone (**5**), 6α-hydroxydemethylcryptojaponol (**6**), cyrtophyllone B (**7**) and 14-deoxycoleon U (**8**). These known compounds were isolated for the first time from the titled plant. The structures of the new compounds were established on the basis of spectroscopic methods. In addition, the isolates were evaluated for their antibacterial activity. Of them, a new compound **2** showed strong activity against Gram-positive bacteria, *Staphylococcus aureus, S. epidermidis, Enterococcus faecalus* and *Micrococcus luteus*. For example, inhibition zones of a new compound **2** and kanamycin, a positive control, were 27.9 and 24.8 mm, respectively, at the same dose of 25 μg/disc against *M. luteus*.

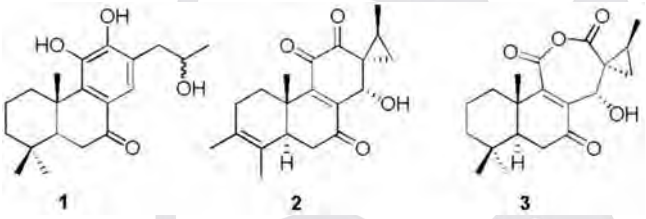

Fig. 1

Acknowledgements: *The authors are grateful to the Science and Technology Foundation of Mongolia for partial financial support.* **References:** [1] Yong-Hong Zhang et al. (2000) Pharmazie 55(11): 845 – 847. [2] Sebastien Hannedouche et al. (1999) Phytochemistry 51:767 – 769. [3] Ligaa U et al. (2006) Medicinal Plants of Mongolia used in Western and Eastern Medicine. JKC printing, Ulaanbaatar, Mongolia.

PA5 HPLC-quantification of yangambin and cytotoxicity of *Ocotea duckei* extract against fibroblast cell line L929 for studies in neglected diseases such as Leishmaniasis

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Leishmaniasis is a neglected poverty-related disease and, currently, is considered to be endemic in 88 countries. The high price and the low access to medicines have led the research for other substances to treat this disease. The important activity of several lignans, especially yangambin, has been observed recently and the *Ocotea duckei* plant extract, from Brazilian biodiversity, has shown promising activity against *L. amazonensis* and *L. chagasi*. Therefore, the objective of this study was to quantify yangambin by HPLC and to obtain a *screening* of the cytotoxicity of this extract in a fibroblast cell line for further *in vivo* studies. The quantification of yangambin in the plant extract was performed using HPLC (C18 column and MeCN:H_2O (50:50) as mobile phase), using a yangambin standard. Mouse fibroblasts (L929) at 5×10^4/mL were inoculated in 96 well microplate. After 24 hours, they were added with a solution containing the extract (6.25 – 200 μg/mL). At specified periods (30 minutes, 8 h and 24 hours) the culture medium was discarded and the coloration was performed with neutral red. The HPLC assay demonstrated that the extract contained 314,6 mg yangambin per gram dry weight. Regarding toxicity, the 6.25 – 200 μg/mL concentration range studied demonstrated not to be cytotoxic to the fibroblast cell line. Researches have pointed that the IC_{50} for *L. amazonensis* and *L. chagasi* is 143,74 μg/mL and 135,66 μg/mL, respectively, and the inhibition were higher than Glucantime® [1]. Other studies [2] demonstrated that concentrations of pure yangambin (50 and 65 μg/mL), changed the morphological and physiological promastigotes forms. Knowing this and based on the fact that the higher concentration tested was not cytotoxic (200 μg/mL) and it contains 62,92 μg/mL of yangambin, the *Ocotea duckei* extract may be an interesting object for further studies. **References:** [1] Monte-Neto *et al.*; Z. Naturforsch. 62c, 348 – 352 (2007) [2] Monte Neto *et al;* Experimental Parasitology 127, 215 – 221 (2011)

PA6 Anti-Mycobacterium tuberculosis activity of eupomatenoid-5 from *Piper regnellii*

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Tuberculosis (TB) is a disease caused mainly by *Mycobacterium tuberculosis*, which has been widespread since ancient times and remains an important public-health problem, especially in developing countries. One worrying factor in TB treatment is the prevalence of drug-resistant strains, and hence there is an urgent need to develop new anti-TB drugs. The interest in natural products, especially the search for new active principles, has stimulated to invest in studies involving plants. *Piper regnelli* var. *pallescens* is popularly known as caapeba or pariparoba in Brazil. The leaves and roots are used as extracts, infusions or plasters to treat wounds, swellings and infections. The present study determined the anti-*M. tuberculosis* activity and cytotoxicity of eupomatenoid-5. The extraction by supercritical fluid (SFE-CO_2) from leaves of *Piper regnellii* var. *pallescens* was conducted at a temperature of 60 °C and pressure of 24.4 MPa. The chromatographic column purification afforded the isolation of the neolignan eupomatenoid-5, which was identified by ^{1}H NMR and comparison with literature data. Anti-*M. tuberculosis* activity was evaluated using a resazurin microtiter assay plate (REMA) to determine the MIC. The cytotoxicity assay was carried out in macrophages J774G.8 by sulforhodamine B colorimetric assay. Anti-*M. tuberculosis* activity of eupomatenoid-5 and the cytotoxicity exhibited very good activity (MIC of 1.9 μg/mL) and selectivity index (SI = 20). Eupomatenoid-5 is a potential compound for future investigations in anti-TB drugs development. Further studies are being conducted to understand the mechanism of action of this promising compound as well as the synergistic activity with conventional treatment.

PA7 Antimalarial activity of two spray dried formulations of *Bildens pilosa* L. based on Brazilian ethnopharmacology

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Bidens pilosa L. (Asterarecae), a plant native of South America is employed in the Amazon region, for the treatment of malaria. This biological activity, seem to be associated to the presence of polyacetylenes and phenolic compounds as flavonoids (Brandão et al., 1997). According to World Health Organization, each year, occurs 219 million cases of malaria in the world resulting in about 660.000 deaths, mostly in children of low income countries (WHO, 2012). Medicines based on phytopharmaceutical preparations for treatment of endemic diseases such as malaria are interesting strategies justifying the research in this area. The spray drying technique have been successfully applied for the production of solid delivery systems containing plant extracts. The aim of this work was to evaluate the *in vivo* antimalarial activity of two spray dried formulations prepared with the hydroethanolic extract of roots of *B.*

pilosa employing β-cyclodextrin and Aerosil:Microcrystalline celulose (MC) as carriers. Antimalarial activity was performed in mice infected with *Plasmodium berghei,* NK-65 by an approved ethical protocol. The presence of flavonoids and polyacetylens was monitored by high performance liquid chromatography. Results showed that the formulation containing Aerosil®:MC increased in 75% the mice survival, at a concentration of 150 mg/kg, the formulation containing β-cyclodextrin also increased the mice survival at all the doses tested being the concentration of 50 mg/kg the most effective with a 50% survival. Both formulations reduce parasitemia the 5th day compared with the control group. The preparations developed can be used as adjuvants for the treatment of malaria. **References:** [1] Brandão, M.G.L. et al. Journal of Ethnopharmacology, v. 57, p. 131 – 138, 1997. [2] World Health Organization, World Malaria Report 2012, available online http://www.who.int/topics/malaria/en/ **Acknowledgments:** FAPESP (Process No. 2012 9890 – 6)

PA8 UFLC-ESI-MS-based profiling, cytotoxicity and antileishmanial activity of *Rhodostemonodaphne crenaticupula*

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Rhodostemonodaphne is a neotropical genus of lauroid shrubs and trees. In Colombia there are ten species. As part of our research on Lauraceae plants, the present study is directed to the phytochemical exploration, and cytotoxicity and antileishmanial evaluation of *Rhodostemonodaphne crenaticupula* Madriñan. Leaves, wood and bark of this plant were collected in Boyacá, Colombia. Plant material was separately dried, grounded and macerated with ethanol. The chemical profile was determined by UFLC-ESI-MS analyses of the obtaining extracts. They were also tested against promastigotes of *Leishmania panamensis*, J774 murine macrophages and THP-1 monocytes in order to examine the extracts' ability and security. Different chromatographic techniques resulted in the isolation of six aporphine alkaloids from the ethanolic extract from leaves, whose chemical structures were elucidated by spectroscopic methods. The chemical characterization of the remaining ethanolic extracts was achieved with UFLC-ESI-MS techniques, using the isolated compounds as standards. All ethanolic extracts were very rich in aporphine-related compounds, indicating that these kind of metabolites might be the bioactive compounds in the antileishmanial activity (EC_{50}= 5 – 50 μg/mL). In order to support this, isolated alkaloids are currently being evaluated against parasites. Bark extract from *R. crenaticupula* was found to be the most potent antileishmanial. Additionally, extracts exhibited low cytotoxicity against J774 and THP-1 cell lines (IC_{50}= 100 – 200 μg/mL) and showed good selectivity indexes (SI > 5). A bioguided fractionation leading to obtain the bioactive aporphine alkaloids is currently in process.

PA9 Antileishmanial and cytotoxic activities of three plants used in colombian folk medicine

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As part of our research on medicinal plants traditionally used in folk medicine in Casanare (Colombia), some important species were selected for further studies through phytochemical exploration. In this study, we investigate the phytochemical profile, antileishmanial and cytotoxic activities of three selected plants (*Bowdichia virgilioides, Jacaranda obtusifolia,* and *Hymenaea courbaril*). These plants are commonly used in the above-mentioned region to treat different infectious diseases. Leaves from test plants were collected in Casanare, Colombia, dried, grounded and macerated with ethanol. The chemical profile was determined by UFLC-DAD analysis and by measuring total phenolic (TP) and total flavonoid (TF) contents. They were also tested against promastigotes of *Leishmania panamensis*, J774 murine macrophages and THP-1 monocytes in order to examine a preliminary extracts' ability and security in a screening platform. The TP and TF contents (> 30 mg gallic acid equiv/g DE and > 3 mg quercetin equiv/g DE, respectively) were found to be reasonable. Chemical characterization of the ethanol extracts was achieved by UFLC-DAD employing a validated method for profiling herbal extracts using standards. All extracts were found to be enriched in flavonoid-related metabolites. Antileishmanial activity of extracts exhibited relatively high EC_{50} values (20 – 100 μg/mL). The extract from *Hymenaea courbaril* was the most active (EC_{50}= 20.1 μg/mL). Additionally, a low cytotoxiciy against J774 and THP-1 was determined for all extracts (IC_{50} > 600 μg/mL, selectivity indexes (SI) > 8). Although the activity against promastigotes is quite low, and tests with the more relevant amastigotes must follow, these results might be related to the traditional use of these plants to treat infections. Therefore, the evaluation of test extracts against amastigotes and a bioguided fractionation to obtain bioactive compounds is currently in progress.

PA10 8-O-4'-connected neolignans as antileishmanial agents: an exploration by molecular docking

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A tropical infectious disease that still remains as a major cause of morbidity and mortality is Leishmaniasis produced by parasites of the genus Leishmania. Discovery of new drugs to control and treat that disease is practically incomplete. Thus, as part of our studies related to the search for therapeutic alternatives against Leishmania parasites, a molecular docking study was performed on a set of 8-*O*-4'-neolignan-like molecules in order to analyze the binding of this kind of neolignans on active sites of three main enzyme drug targets: farnesyl pyrophosphate synthase, trypanothione synthetase and *N*-myristoyltransferase. In order to observe the mode of binding, Autodock Vina was used to dock the most stable conformers of fifteen 8-O-4'-neolignans within the active site of Leishmania enzyme-based drug targets. Test structures were optimized at DFT level using B3LYP functional and 6 – 31G basis set. Stability of enzyme complexes between enzymes and neolignans were investigated through Vina scores and selected active site residues interactions. Good Vina scores were obtained for enzyme-ligand complexes interactions at different levels. Most stable conformer of neolignan **1** was found to exhibit the best Vina-score. In addition, the results indicated that the best poses were found to be different for each test compound as well as the active site residues involved into the ligand-enzyme binding. Residue-ligand interaction profile was correlated with Vina scores, exhibiting important structure-interaction relationships useful in further studies. Binding modes of 8-O-4'-neolignans into enzymes using ligand-protein docking were found to be different. This is the first study to provide an explanation at atomic level of the binding of this kind of neolignans into the above-mentioned enzymes. Further structural optimization of 8-O-4'-neolignans moieties should be considered in order to design antileishmanial agents.

PA11 *In silico* methods to select biologically active anti-protozoan natural products

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Neglected diseases (ND) caused by tropical protozoan parasites threaten millions of people in the world. Despite the efforts from academia, government authorities and pharma companies, the available marketed drugs are insufficient. Natural products (NP) comprise a rich source of compounds that may lead to new drugs against ND. In 2011, the Research Network Natural Products against Neglected Diseases (ResNet NPND)[1] has been established as an international research initiative to make efforts against this global threat. In this work, we describe the use of in silico methods to find NP hits against protozoan parasites. A ResNet NPND data bank with *ca.* 1,000 chemical structures of anti-parasitic NP and their biological data has been created[2]. 2D/3D structure descriptors were calculated and non-relevant variables were eliminated by correlation matrix followed by linear forward selection. The *in vitro* activity data of 513 chemical structures was grouped into four different classes against the following parasites: *Plasmodium falciparum* (A; 291 structures), *Trypanosoma brucei* (B; 63), *T. cruzi* (C; 98) and *Leishmania donovani* (D; 61). Random Forest (RF) and k-Nearest Neighbors (k-NN), machine-learning tools used to classify objects according to their classes, were used for a multi-class classification of the 513 chemical structures

of NP according to their biological activities (A-D). The cross-validated models using 2D descriptors showed an accuracy of 70 – 90% and good statistical significance (Kappa statistic and ROC area). Additional 2,948 chemical structures of NP from Analyticon Discovery GmbH (Germany) with unknown biological activities were used as external data set. The RF and k-NNN models were suitable to predict new NP hits against multiple targets that can be further investigated by docking and biological assays. **Acknowledgements:** CAPES and BMBF. **References:** [1] www.uni-muenster.de/ResNetNPND [2] Schmidt TJ et al. *Curr. Med. Chem*., 2012, 19, 2128 – 2175 and 2176 – 2228.

PA12

In silico screening of natural product databases reveals new potential leads against neglected diseases

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Infections with "protozoan" parasites, such as Malaria, Leishmaniasis or Trypanosomiasis cause tremendous suffering in wide parts of the world. Due to a lack of novel therapeutics, more research in this field is urgently required. Many natural products (NPs) have shown impressive activity while their mechanisms of action are mostly unknown. In this *in silico* study [1], structures of enzymes of major protozoan pathogens and NP databases have been studied to reveal new leads against these neglected diseases. Structures of NPs that had been tested against at least one major protozoan pathogen were extracted from literature (Pubmed [2]). The search was focused on phenolic plant constituents. Optimized 3D structures were collected in a database (1712 compounds; PheDB). A second database with 928 NPs published in [3] was created accordingly (NPDB). These two collections of potentially active substances were used for virtual screening. Structures of potential protein targets [3] were acquired from the Protein Databank [4]. Co-crystallized ligands were used to create pharmacophore queries which were employed for a virtual screening (VS) of the databases. The hits resulting from this VS were docked into the relevant enzymes' active centers. Docking-scores were compared to those of the co-crystallized ligands (CCL). About 40 enzymes were studied so far yielding promising results (better docking scores than CCL) for NPs of various classes (Table 1). Experimental validation, i.e. enzyme inhibition tests, have been initiated.

Tab. 1: Results of the virtual screening of different protozoal proteins

	T. brucei	T. cruzi	Leishmania ssp.	P. falciparum
Number of investigated	10	9	13	13
Number of hits (PheDB)	71	235	133	162
Number of hits (NPDB)	85	101	403	123
Most promising target	TbIGNH	TcHPRT	LmexGAPDH	PfENR
PDB-entry number	3FZ0	1TC1	1N1G	2OL4
PRS (%)* of the most promising ligand	154	174	222	146

*PRS (%)= Protein relevance score: Ratio in % between docking score for best docking ligand and co-crystalized ligand

References: [1] For all calculations: Molecular Operating Environment 2011.10 (MOE), Chemical Computing Group, Montreal, Canada, http://www.chemcomp.com [2] http://www.ncbi.nlm.nih.gov/pubmed/ [3] Schmidt, T.J. *et al.* Curr Med Chem. 2012; 19: 2128 – 75 and 2176 – 228. [4] http://www.rcsb.org/pdb/home/home.do This work is part of the activities of ResNetNPND: http://www.uni-muenster.de/ResNetNPND/ Support of CCG, Montreal, is gratefully acknowledged

PA13

In vitro cytotoxic activity of *Derris scandens* stem extracts against lung cancer cells and their anti-oxidant and anti-microbial effects

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Derris scandens (Roxb.) Benth (DS), as Tao-Wan-Priang is popularly used in Thai traditional medicine as expectorant, antitussive and cancer cure [1]. Therefore, the objectives of this study were to investigate the cytotoxic activity of DS extracts against three types of lung cancer cell lines, such as human large cell lung carcinoma cell line (COR-L23), human lung adenocarcinoma cell line (A549) and human lung squamous carcinoma cell line (NCI-H226), and comparison with activity on normal lung cells, i.e. human lung myofibroblast cell line (MRC-5) by SRB assay[2]. Antioxidant activity by DPPH assay and anti-microbial activity were also tested [3,4]. The results showed that 95% and 50% ethanolic extracts exhibited cytotoxic activity against three lung cancer cell lines COR-L23, A549 and NCI-H226. The 95% ethanolic extract was the most active against COR-L23 (IC_{50}= 25.20 ± 3.33 µg/ml). In addition, the 50% ethanolic extract was the most active against A549 and NCI-H226 (IC_{50}= 6.98 ± 3.45, 18.50 ± 0.92 µg/ml, respectively). In contrast, the water extract showed low cytotoxic activity against the three types of lung cancer cell lines. All three extracts showed low anti-oxidant activity in the DPPH assay with EC_{50} values of 41.32 ± 3.73, 61.91 ± 0.81 and > 100 µg/ml, respectively, while BHT as a positive control exhibited an EC_{50} value of 10.66 ± 0.97 µg/ml. The 95% ethanolic extract showed higher anti-microbial activity against *B. subtilis* than against *S. aureus* (MIC = 20 and 40 µg/ml, respectively). The 50% ethanolic extract showed as the same effects. **References:** [1] Kuptniratsaikul V et al. (2011) Journal of Alternative and Complementary Medicine 17(2): 147 – 153. [2] Skehan P et al. (1990) Journal of National Cancer Institute 82: 1107 – 1112. [3] Yamazaki K et al. (1994) Journal of Chemical and Pharmaceutical Bulletin. 1663 – 1665. [4] Lorian V. (1996) Antibiotics in Laboratory Medicine, 3 rd Ed. Williams and Wilkins, Baltimore.

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PEG2 inhibitory compounds from *Dioscorea membranacea* roots

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The roots of *Dioscorea membrancea* have been used as an antiinflammatory drug in Thai traditional medicine [1]. Bioassay-guided fractionation was used to isolate the anti-inflammatory ingredients by testing PEG2 inhibitory effect on RAW264.7 macrophages [2]. Dioscorearide B (1), dioscoreanone (2) and two dihydrophenantrene compounds, 2,4 dimethoxy-5,6-dihydroxy-9,10-dihydrophenanthrene (3) and 5-hydroxy-2,4,6-trimethoxy-9,10-dihydrophenanthrene (4), were isolated. 4 showed the highest PEG2 inhibitory effect, followed by 1 and3 (IC_{50}= 7.65, 20.83 and 28.56 µM, respectively). Dioscoreanone had no PEG2 inhibitory effect. Indomethacin as a positive control showed inhibitory effect on PEG2 release with an IC_{50} value of 2.8 µM. **References:** [1] Pongboonrod, S (1976) Medicinal plants of Thailand. Kasem Bundit, Bangkok, Thailand pp180. [2] Tewtrakul S and Itharat A (2007) J ethnopharmacol, 105,312 – 315.

PA15

Bioprospecting using a statistical approach applied to an ethnopharmacological database on 'snakebite-plants' – the genus *Piper*

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The present study was undertaken to investigate whether statistical evaluation of ethnobotanical data enhances the selection of species with pharmacological activities. A database was created for plants used to treat snakebites worldwide, which included 164 plant families, 883 genera and 1521 species (1). Five countries with a high number of entries, representing different cultures, geography and floristic zones were selected: Brazil, Nicaragua, Nepal, China and South Africa. The datasets were analysed by regression and binominal analysis in to see if any „snake-plant family/genera" was overrepresented in the respective traditional medicinal systems relative to the abundance in the local flora (1). The only genus recognized as a positive outlier was *Piper*. 11 species of *Piper* were obtained from the Copenhagen Botanical Garden. Water, ethanol and chloroform extracts were prepared. The extracts were tested for inhibition of hyaluronidase, phospholipases and proteases in microplate-based enzyme assays, using *Bitis arietans* venom as enzyme source. These enzymes are involved in the tissue necrosis following a snakebite. The results showed a moderate activity of chloroform extracts of *P. arboretum* and *P. sylvaticum* in the hyaluronidase assay. The ethanolic extract of *P. cernuum* showed moderate inhibition of phospholipase A2. The water extracts of all *Piper* species showed some inhibition

of protease. The chloroform extract of *P. sylvaticum* showed high inhibition of protease with an IC_{50} of 29 µg/ml. The ethanolic extracts of *P. sylvaticum* and *P. nigrum* also showed moderate inhibition of protease. All the tested species of the selected genus did show some activity. This indicates that statistical evaluation of ethnobotanical data might be merited before undertaking a pharmacological study. **References:** [1] Molander M, Haris Saslis-Lagoudakis C, Jäger AK, Rønsted N. Cross-cultural comparison of medicinal floras used against snakebites. J Ethnopharm 2012; 139: 863 – 872

PA16 Inhibitory effect of active peptide from oyster hydrolysate on Angiotensin-I Converting Enzyme (ACE)

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Heart disease is the leading causes of death in many countries and high blood pressure has been one of the main risk factors for it. [1] Angiotensin-I Converting Enzyme (ACE) has an important physiological role in the regulation of blood pressure. [2] The objective of this research was to study the ACE inhibition effect of oyster hydrolysates fraction (OH) and isolate active peptide. We investigated the effects of oyster hydrolysates fraction and active peptide on the blood pressure levels in stroke-prone spontaneously hypertensive rats (SHRSPs). Thirteen-week-old SHRSPs were assigned to four groups; the control group, positive control group (Val-Tyr), OH group, and isolated active peptide groups. Administration of Val-Tyr (50 µg/kg), OH (100 mg/kg) and isolated active peptide (50 µg/kg) decreased the maximum blood pressure at 9 (29.7%), 9 (37.9%) and 6 hr (12%), respectively. The blood pressure levels in treated group were maintained until 24 hr after administration. Furthermore, we measured ACE activity in the serum, aorta and kidney. In the serum and aorta, the SHRSPs showed higher ACE activity than the treated groups. These results suggest that OH and the isolated active peptide can be considered as a potential source for antihypertensive functional foods or drugs. **References:** [1] James R.S. et al. (2001) Hypertension 37: 1053 – 1059. [2] Tetsuya S. et al. (2004) Hypertension 43: 1003 – 1010.

PA17 Profiling the antiplasmodial polyphenolic fraction of *Anogeissus leiocarpus* leaves by LC/PDA/ESI-MS/MS

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Leaves decoctions of *Anogeissus leiocarpus* (Combretaceae) have long been used in the African traditional medicine to treat malaria, trypanosomiasis, dysentery and helminthiasis.[1, 2] As part of our on-going activity within the "Research Network Natural Products against Neglected Diseases" (ResNet NPND) we subjected the polyphenolic ethyl acetate fraction which is associated with the antiplasmodial activity of *A. leiocarpus* leaves to chemical profiling with LC/PDA/ESI-MS/MS. The 80% methanolic extract of leaves of *A. leiocarpus* was sequentially fractionated with petroleum ether, chloroform and ethyl acetate. The aforementioned fractions were tested against chloroquine sensitive *Plasmodium falciparum* NF54 with chromogenic assay involves the biological reduction by viable cells of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium (MTS) at two different concentrations 10 µg/ml and 5 µg/ml. The ethyl acetate fraction significantly inhibited the malaria parasite (99%) at 10 µg/ml with no sign of toxicity. RP-HPLC-DAD coupled with tandem mass spectrometry performed on the bioactive ethyl acetate fraction led to the identification of six derivatives of ellagic acid, mainly methyl ether and its glycosides besides four quercetin conjugates and a kaempferol glycoside as well as two stilbenes, namely E-viniferin and methoxy E-viniferin which were not previously reported from this genus. **References:** [1] Neuwinger, H.D. 2000. Press Stuttgart, Germany: 46. [2] Okpekon, T.; Yolou, S.; Gleye, C.; Roblot, F.; Loiseau, P.; Bories, C.; Grellier, P.; Frappier F.; Laurens, A. and Hocquemiller, R., 2004. *Journal of Ethnopharmacology*, 90: 91 – 97. [3] Adigun, J.O.; Amupitan, J.O. and Kelly, D.R., 2000. *Bull. Chem. Soc. Ethiopia*, 14(2): 169 – 174.

PA18 Soulamarin isolated from *Calophyllum brasiliense* (Clusiaceae) induces plasma membrane permeabilization of *Trypanosoma cruzi* and mytochondrial damage

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In the course of selection of novel drug candidates for Chaga's disease from Brazilian flora, we undertook the study of stem bark from *Calophyllum brasiliense* (Clusiaceae)[1,2]. After extraction, the MeOH extract was partitioned between MeOH:H_2O and EtOAc. The organic phase displaying anti-*Trypanosoma cruzi* activity and was subjected to Sephadex LH-20 (MeOH), to yield nine fractions (A – I). Through bio-guided fractionation, the activity was detected at fraction C, which was subjected to silica gel column (hexane-EtOAc) resulting in four fractions (C1 – C4). Since the activity was detected in fraction C2, this material was purified using preparative TLC to afford soulamarin (Figure 1), a previously isolated coumarin from *C. soulatrii*[3]. The structure of this compound was established on the basis of spectroscopic data, mainly NMR and MS. Soulamarin showed activity against trypomastigotes of *T. cruzi* with an IC_{50} value of 85.3 µg/mL and a similar IC_{50} value to benznidazole (114.6 µg/mL). No hemolytic activity could be detected up to 150 µg/mL. By using the fluorimetric vital dye SYTOX Green, soulamarin induced permeabilization of plasma membrane when compared to untreated group. Spectrofluorimetric data using MitoTracker Red, demonstrated that soulamarin also induced a strong depolarization of the mitochondrial membrane potential, reducing the fluorescence intensity by 97% when compared to untreated group. These data suggest that the lethal effects of soulamarin in T. cruzi involve damages to plasma membrane of the parasite, which may have contributed to the mitochondrial disturbance and cell death. Considering the unique mitochondrion of *T. cruzi*, secondary metabolites of plants as soulamarin may contribute as scaffolds for the design of novel and selective drug candidates for neglected diseases. Support: FAPESP and CNPq. **References:** [1] ABE F et al. *Biol. Pharm. Bull*. 2004, 27,141 – 143. [2] NOLDIN VF et al. *Quím. Nova* 2006, 29, 3, 549 – 554. [3] EE GCL et al. *Molecules* 2011, 16, 9721 – 9727.

PA19 *Ocotea notata* extracts: chemical profile and *in vitro* antimycobacterial activity

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The *Ocotea* genus (Lauraceae) is mainly distributed in tropical and subtropical regions. Some species of this genus as *O. puberula* and *O. quixos* have been described in literature by exhibiting antibacterial activity. Tuberculosis (TB) is a major challenge worldwide, showing high rates of co-infection with mycobacteria. Our goal was to evaluate the inhibitory effect of *O. notata* extracts, from Jurubatiba Shoal against TB mycobacteria bacillus. Aerial parts of *O. notata* were dried, pulverized and soaked for 7 days. The crude extract obtained was subjected to a liquid-liquid partition by using solvents with different polarities and subsequently monitored on thin layer chromatography (TLC). For the ethyl acetate and buthanol fractions there were used butanol: acetic acid: water (BAW) 8:1:1 as mobile phase and NP-PEG as chromogenic reagent. The TLCs were observed in a dark chamber under UV light at

wavelengths λ_{254} and λ_{365} nm which indicated the presence of flavonoids. The fractions were also analyzed by HPLC-DAD which confirmed the presence of phenolic skeletons by characteristic UV spectra (210, 256 and 352 nm). The hexane fraction was analyzed by GC-MS being detected the presence of sesquiterpenes, among them santalol and spatulenol as majors. The biological assay was initially performed by using *Mycobacterium bovis* BCG, which was distributed in 96 well plate with culture medium 7H9+ADC, 1 x 10^6 CFU/well. The *O. notata* samples were added at 0.8; 4; 20 and 100 µg/mL and rifampicin used as control. The plate was incubated for 7 days and mycobacterial growth quantified by the MTT method (n = 3, Statistical: ANOVA). At the concentrations tested it was observed that the leaf crude extract was able to inhibit 81.9 ± 6.48% at 100 µg/mL and the hexane and ethyl acetate fractions to inhibit 72.9 ± 9.72% and 48.5 ± 0.29% at 100 µg/mL, respectively. These results suggest that the constituents present in the active fractions may be promising to the search for new agents against *M. bovis.*

PA20 *Ampelozizyphus amazonicus* inhibit proliferation and release of *Trypanosoma cruzi* in murine macrophages in vitro

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Ampelozizyphus amazonicus Ducke (Rhamnaceae) is an Amazonian medicinal plant popularly known as "saracura-mirá", from which barks and roots an aqueous drink can be prepared. In an ethnopharmacological study conducted in the "quilombola" communities of Oriximiná (PA), Brazil, we learnt the plant is used in the treatment of liver disorders, and as a tonic, among other uses. A trypanocidal activity for an extract of this plant has been demonstrated in literature. In this work, we investigated the effect of *A. amazonicus* on *Trypanosoma cruzi* in murine macrophages. Barks of the plant were collected and extracted twice with boiling water, filtered and dried to obtain a powder (SART). Peritoneal macrophages from BALB/c mice were infected with metacyclic forms of the *Trypanosoma cruzi* clone Dm 28c (3 parasites per macrophage) and some cultures were treated with *A. amazonicus*. Three days after infection the number of amastigotes forms was evaluated. The numbers of trypomastigote forms were determined by counting of the supernatant in a hemocytometer after 7 and 9 days of culture. In addition, we tested whether nitric oxide was involved in the mechanism of resistance to infection in our *in vitro* model. To address that hypothesis, peritoneal macrophages were stimulated with LPS, INF-γ and treated with *A. amazonicus,* and nitric oxide concentration was measured. Statistical analysis was performed using the Student t-test for independent samples, with the level of significance set at $p < 0,05$. Our results have shown that *A. amazonicus* reduces significantly the replication of amastigotes forms (35% reduction), the release of trypomastigotes forms (75% reduction) in murine macrophages infected by*Trypanosoma cruzi in vitro,* and that this effect does not depend on nitric oxide production. Taken together, our results suggest that *A. amazonicus* is a promissing natural product with important trypanocidal action in intracellular infection. Support: FAPERJ and CNPq.

PA21 Antioxidant and radical scavenging activity of *Limonia acidissima* (Linn) extract

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Free radicals induce numerous diseases by lipid peroxidation, protein peroxidation and DNA damage. It has been reported that numerous plant extracts have antioxidant activities to scavenge free radicals. In the present study, the antioxidant properties of crude extract of *Limonia acidissima (linn)* were examined, using different *in vitro* analytical methodologies, such as total antioxidant activity determination by ferric thiocyanate, hydrogen peroxide scavenging, 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS + radical cation) radical cation scavenging activity and superoxide anion radical scavenging by riboflavin-methionine-illuminate system. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol were used as the reference antioxidant radical scavenger compounds. The crude extract inhibited 94.50% peroxidation of linoleic acid emulsion at 20 µg/ml concentration, while the standard antioxidants BHA, BHT and α-tocopherol indicated an inhibition of 93.75, 96.66 and 83.33% at 60 µg/ml concentration, respectively. The hydrogen peroxide radical, DPPH radical, ABTS + radical cation(s) and superoxide anion radical scavenging activities of crude extract were also compared to BHA, BHT and α-tocopherol as references antioxidant compounds. The present study shows that the crude extract is an effective natural antioxidant component.

PA22 Metabolomics studies of endophytic metabolites from Malaysian mangrove plant in the search for new potential antibiotics

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Currently, endophytic fungi have been explored not just for their ecological functions but for their secondary metabolites as a new source of these pharmacologically active natural products. Accordingly, many structurally unique and biologically active compounds have been obtained from the cultures of endophytic fungi. The fungal strains *Aspergillus aculeatus* and *Lasiodiplodia theobromae* were isolated from the stem of the mangrove plant *Avicennia lanata c*ollected from the East coast of Peninsular Malaysia in Terengganu Province, Malaysia. The fungi were taxonomically identified according to their morphological characteristics as well as by DNA amplification and sequencing of the ITS region. Prior to this study, metabolomics has been applied to identify and optimize the production of bioactive secondary metabolites in both fungi at different growth stages and culture media. Metabolomic studies were afforded by both high resolution mass spectrometry and NMR spectroscopy. Metabolomic profiling data was processed by utilizing the quantitative expression analysis software Mzmine 2.10 coupled with the Antimarin database for dereplication studies. SIMCA P+ 13.0 was used to prove that the optimization models were statistically sound. Respective fungi were then later scaled up either in rice-solid and liquid culture media. Crude extracts were fractionated using several high-throughput chromatographic techniques and subjected to bioactivity-guided isolation work for anti-trypanosomal active metabolomes. Structure elucidation of isolated secondary metabolites was achieved using 2D-NMR and HRESI-MS.

PA23 LC-MS, GC-MS and *in vitro* pharmacological analysis of *Clematis vitalba* L. upper parts

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The aerial parts and roots of *Clematis vitalba* L. (Ranunculaceae) are traditionally used as remedy for inflammatory diseases. In this study we analyzed fresh and dried flowers, leaves and stems as well as fresh fruits. These plant organs were extracted with three different solvents (ethanol, acetone and dichloromethane (DCM)) and tested for NF-κB inhibition and PPARbeta/delta activation in HEK293 cells via luciferase-based reporter gene assays. The ethanol and the acetone extract of the fruits showed the highest activity in both assays, followed by the DCM extracts of the fresh flowers and fruits (see table 1). Phytochemical analysis of fruit extracts using LC-DAD-MS^n and GC-MS analysis showed very similar chemical profiles of the ethanol and acetone extracts with

flavonoids and triterpenes as the major constituents. In the DCM extract compounds like a phenylalanin derivative and sterols dominated the LC-MS profile. In the fresh flower DCM extract only one main peak dominated the LC-MS chromatogram. It was tentatively identified as the isovitexin derivative vitalboside.

Tab. 1: Pharmacological activities of *Clematis vitalba* L. active extracts

Plant parts/ pharmacological assay	Fresh fruits			Fresh flowers	Positive control
	EtOH	Acetone	DCM	DCM	
NF-κB inhibiton (% inhibition at 50 µg/ml)	98.6%	98.0%	58.5%	78.4%	75.8% (Partenolide 5 µM)
PPARbeta/delta activation (fold activation at 10 µg/ml)	5.41 ± 1.48	4.81 ± 1.26	2.30 ± 0.43	1.98 ± 0.34	16.70 ± 4.01 (GW0742 20 nM)

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PA24 Evaluation of the *in vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants from the Arabian Peninsula

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Today over one billion people worldwide are at risk for tropical diseases caused by parasitic organisms. The World Health Organization (WHO) now classifies many as neglected tropical diseases, having an enormous impact on socioeconomic development and quality of life at all levels particularly in developing countries. Malaria, leishmaniasis and human African trypanosomiasis are still major public health problems in need for new and more effective drugs. The aim of this study was to exploit traditional healer knowledge and evaluate *in vitro* antiprotozoal activity of twenty-five medicinal plants collected from Saudi Arabia and the island Soqotra. The plants were extracted with methanol and screened for their *in vitro* antiprotozoal activity against erythrocytic schizonts of *Plasmodium falciparum*, intracellular amastigotes of *Leishmania infantum* and *Trypanosoma cruzi* and axenic *T. brucei* trypomastigotes. To assess selectivity, cytotoxic activity was determined against MRC-5 cells. Criteria for activity were an IC_{50} < 10 µg/ml. Selective activity was obtained for *Chrozophora oblongifolia* (Del.) A. Juss. ex Spreng., *Dracaena cinnabari* Balf. f., *Ficus ingens (Miq.), Hypoestes pubescens* Balf. f., *Lavandula dentata* L., *Plectranthus barbatus* Andr. and *Punica protopunica* Balf. f. against *P. falciparum* (IC_{50} 2 – 8 µg/mL) while *Dracaena cinnabari, Euriendra balfourii* Cogn. & Balf. f., *Grewia erythraea* L. and *Vernonia leopoldii* Vatke displayed activity against the three kinetoplastid parasites (IC_{50} < 10 µg/mL). *Acridocarpus socotranus* Oliv. was moderately active against *T. brucei* (IC_{50} 3.5 µg/mL). *Ballochia atrovirgata* Balf. f., *Dendrosicycos socotrana* Balf. f., *Dracaena cinnabari* and *Euphorbia socotrana* Balf. f. displayed non-specific inhibition of the parasites related to high cytotoxicity. Acknowledgments: The authors extend their appreciation to the NPST program by King Saud University for funding the work through the project number (10-MED 1288 – 02). The authors gratefully acknowledge that financial support.

PA25 Investigation of the mechanism of action involved in the cell death of *Leishmania amazonensis* treated with eupomatenoid-5, an isolated compound from *Piper regnellii* var. *pallescens*

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Leishmaniasis is a tropical disease caused by parasite *Leishmania.* Twelve million people are currently infected with *Leishmania* and up to 350 million people are at risk of infection [1]. Drugs available for the treatment of this infection have many limitations including resistance and toxicities [2]. It is known that plants represent an important source of potential biological agents. In a previous work, we described the antileishmanial activity of eupomatenoid-5, an isolated compound of *Piper regnellii* var. *pallescens* [3]. The aim of this work was to investigate the mechanism of action induced by this compound in the cell death of *L. amazonensis*. For this, promastigotes were treated with 30, 85 or 170 µM eupomatenoid-5 and incubated with Rh123 or annexin-V FITC/PI. Eupomatenoid-5 caused a decrease in Rh123 fluorescence of 76.8% for 30 µM and 95.9% for 85 µM indicating mitochondrial depolarization. Moreover, at higher concentrations (85.0 and 170.0 µM), annexin-V fluorescence intensity was increased by more than 30%, indicating phosphatidylserine exposure, an apoptotic marker that is present in the outer leaflet of plasmalemma. Treatment of promastigotes also resulted in 16% and 28% increase in the proportion of cells in sub-G0/G1 phase at 30 and 85 µM, respectively, showing that eupomatenoid-5 induced G0/G1 phase cell cycle arrest, corresponding to DNA fragmentation. It is possible to suppose that the antileishmanial action of eupomatenoid-5 may involve its effect on the mitochondrial function leading to parasite death by apoptosis. **References:** [1] WHO. Leishmaniasis: The Global Trend. http://www.who.int/neglected_diseases/integrated_media leishmaniasis/en/index.html (2012). [2] M. Banerjee et al. *European Journal of Medicinal Chemistry* 55 (2012) 449 – 454. [3] M.C. Vendrametto et al. *Parasitology International* 59 (2010) 154 – 158. **Acknowledgements:** *CNPq, Fundação Araucária, FINEP, and CAPES.*

PA26 Synthetic flavone derivatives as potential new *in vivo* antimalarial agents

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Malaria is a burden causes approximately 660.000 deaths every year, mostly among African children. Since 2000, mortality rates have fallen by more than 25%, indicating that the WHO Global Malaria Program is efficient[1]. However, the emergence of resistance to artemisinin could reverse the trend of decreasing mortality[2]. Consequently, there is an urgent need of discovering new treatments associated with the exploration of novel targets. Thus, we are developing new synthetic antimalarial agents with an original structure inspired by nature. The isolation of an active biflavonoid from *Campnosperma panamense* (Anacadiaceae) led us to the development of simplified synthetic analogs. Structure Activity Relationship (SAR) study is still in progress, but several active compounds have already been synthesized. Nevertheless, the most active compounds *in vitro* are poorly bioavailable and were inactive *in vivo* on the murine model *Plasmodium berghei* ANKA. One compound, MR70, is less active than others (IC_{50}= 1.9 µM, *P. falciparum* strain K1) but exhibits an interesting bioavailability that could potentially induce an *in vivo* activity (plasma level of 8.1 µM two hours after an intraperitoneal injection of 100 mg/kg). Indeed, in preliminary studies conducted on mice infected intraperitoneally by an *inoculum* of 2.10^6 parasites (*P. berghei* ANKA), MR70 enabled to reverse the parasitemia of infected mice by 50% when administered intraperitoneally one hour after parasites inoculation and for 3 more days (4 x 100 mg/kg). Within this context, the SAR study should be pursued in order to increase the antimalarial activity and the bioavailability. Once an active molecule will be satisfactory, we will try to elucidate its mechanism of action, with the hope of discovering a new target. **References:** [1] World Malaria Report, 2012 [2] Phyo, A. P. *et al.* Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. *Lancet* **379,** 1960 – 1966 (2012).

PA27 Antioxidant and cytotoxic properties of *Senna alata* and *Senna podocarpa* leaf extracts

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This study determines the antioxidant and cytotoxic potential of hydromethanolic leaf extract (HMLE) of *Senna alata* and *Senna podocarpa*, which are herbs commonly used in southern Nigeria, to treat a number of diseases (Okpuzor *et al*., 2004; Faruq *et al*., 2010) including sickle cell anemia and diabetes mellitus, which are characterised by oxidative stress (Vincent *et al*., 2004; Nur *et al*.,2011). Hydromethanolic leaf extracts were prepared by reflux, and total phenolics, flavonoids and tannin contents were determined using standard assays (Abdel-Hameed, 2009). The *in-vitro* antioxidant activities of the methanolic extracts were evaluated by assessing the total antioxidant capacity, total reducing power, DPPH radical scavenging activity (DRSA) as well as their DNA protection ability. The WST-1 cytotoxicity test was performed using K562 cell line. The results showed that *Senna podocarpa* HLME had a significantly higher flavonoid, tannin and phenolic content when compared to *S. alata* HLME, but *S. alata* HLME exhibited a significantly higher total antioxidant capacity. The reductive capability of Gallic acid > *S. podocarpa* HMLE > *S. alata* HMLE. The DRSA increased with increasing concentrations of both HLMEs and gallic acid. The DRSA of S. alata HMLE was more pronounced than that of *S. podocarpa* HMLE but weaker than the control gallic acid. The HMLE of *S. podocarpa* exhibited a dose dependent DNA protection activity, while the HMLE of *S. alata* at 10 µg/ml and 1000 µg/ml exhibited pro-oxidant activity, but 100 µg/ml showed DNA protection activity. Cytotoxicity was displayed by the HLME of *S. alata* and *S. podocarpa* and the CC_{50} values were 104.5 ± 3.35 µg/ml and 131.8 ± 1.67 µg/ml respectively. The herbal control drug Nicosan had a CC_{50} value of 115.3 ± 1.99 µg/ml. The hydromethanolic leaf extracts of S. alata and S. podocarpa have potent antioxidant properties, but both extracts were cytotoxic to the K562 leukaemia cell line.

PA28 Ameliorative potential effect of grape seeds extract on testicular damage induced by sodium arsenite in rat

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Inorganic arsenic is a major environmental pollutant with multiple toxic effects in animal and human populations. Acute and chronic arsenic exposure causes marked damage in various organs, including the testes. The present study was aimed to investigate the grape seeds extract effect on sodium arsenite. Adult rats were administered grape seeds extract (GSE) (75 & 150 mg/kg/day), after 30 minutes they intraperitoneally injected with sodium arsenite (8 mg/kg b.w./day) for 60 days. Sodium arsenite significantly reduced testicular sperm count and motility ($p < 0.001$) by 53% and 64%, respectively versus control values. Treatment with GSE increases sperm concentration and motility. Testicular weight, plasma testosterone levels, the activities of glutathione peroxidase (GSH-Px), glutathione (GSH), superoxide dismutase (SOD) and catalase levels significantly decreased, as well as, malondialdehyde (MDA) and nitric oxide (NO) levels significantly increased in testes tissue homogenates of the group treated with sodium arsenite. GSE significantly reversed the reduction of plasma testosterone levels and antioxidant enzyme system toward the normal values in dose dependant manner. Histopathological examination supported the above claims. Microscopic examination of sodium arsenite-treatment rats showed reduction in seminiferous epithelial layer, arrested maturation, damaged Sertoli and leydig cells and perivascular fibrosis were found. The histopathological examination resulted that the combined treatment with GSE and sodium arsenite- markedly reduced these pathological findings. Also, GSE treatment decreased the expression of iNOS, and caspase-3. These results improved that grape seeds extract has a potent protective effect in the treatment of testicular tissue damage caused by sodium arsenite. This effect may be due to the presence of flavonoides and antioxidant properties of GSE and it may be useful in the treatment of testicular toxicity caused by other toxicants.

PA29 Evaluation of antiplasmodial activity of five sudanese medicinal plants on the basis of enoyl-ACP reductase (PfENR) inhibition

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Plasmodium falciparum is the most serious health threat in Sub-Saharan Africa[1]. This situation is further aggravated by the resistance to currently known antimalarials coupled with the lack of an effective vaccine. Therefore, there is an urgent need to discover new viable biochemical targets and biologically active compounds. The discovery of a type II fatty acid biosynthesis pathway (FAS II) in *P. falciparum,* particularly, the enoyl-ACP reductase (PfENR), which catalyses the rate limiting step in each elongation circle, has been recognized and validated as an important target[2,3]. The present work capitalizes on the discovery of new antimalarial molecules based on PfENR inhibition. The alcoholic extracts of five plants commonly used in the Sudanese traditional medicine to treat malaria exhibited certain degree of inhibition of PfENR in concentration less than 250 µg/mL. Bioactivity-guided fractionation revealed that the most potent inhibitors of PfENR were the ethyl acetate fraction of *Acacia nilotica* and *Khaya senegalensis* stem barks followed by their water residue and finally the *Ziziphus spina-christi* root bark with an IC_{50} of 0.87, 3.35, 6.33, 11.68 and 15.62 µg/mL respectively. The very prominent activity of the ethyl acetate fraction of *Acacia nilotica* had encouraged us to further analyze it in order to isolate bioactive compound(s) associated with the aforementioned activity. **References:** [1] World malaria report: 2012. WHO [2] Freundlich, J. S.; Anderson, J. W.; Sarantakis, D.; Shieh, H.-M.; Yu, M.; Valderramos, J.-C.; Lucumi, et al., *Bioorg. Med. Chem. Lett*. 2005, 15, 5247 – 52. [3] Perozzo, R.; Kuo, M.; Sidhu, A. B. S.; Valiyaveettil, J. T.; Bittman, R.; Jacobs, W. R.; et al., *Biol. Chem.* 2002, 277, 13106 – 14.

PA30 Virola plants from colombian amazon: LC-DAD and LC-MS-based chemical profiling and antileishmanial and cytotoxic activities

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Members of genus Virola are well-known by Amazonian native people due to their medicinal properties. Virola is one of the most important genera of the Myristicaceae family. In Colombian Amazon, twenty one species can be found. We selected to investigate the LC-MS-based chemical profile and antileishmanial activity of three taxa (*V. carinata*, *V. elongata* and *V. peruviana*). Leaves, wood and bark of test Virola plants were collected in Guaviare, Colombia, dried, pulverized and subjected to maceration process with ethanol. Phytochemical profile was determined by measuring total phenolic and total flavonoid and by LC-DAD and LC-MS analysis of the resulting nine extracts for comparative drives by chemometrics through principal component analysis (PCA). In order to explore the effectiveness and security of the extracts as parasitocide agents, extracts were then tested against extracellular *Leishmania panamensis* parasites (promastigotes) and two cell lines (J774 murine macrophages and THP-1 monocytes), respectively. High contents of total phenolics (> 20 mg gallic acid equivalents/g DE) and total flavonoids (> 5 mg quercetin equivalents/g DE) were found. In addition, different chromatographic techniques resulted in the isolation of a novel flavonoid from the ethanolic extract of *V. carinata*, whose chemical structure was elucidated by spectroscopic methods. The chemical characterization of the other ethanolic extracts was achieved with LC-DAD and LC-MS techniques, using the isolated compound from *V. carinata* as standard. All ethanolic extracts were very rich in flavone-like compounds, indicating that this kind of phytoconstituents is possibly responsible for the extracts' antileishmanial activity (EC_{50} < 50 µg/mL). Additionally, extracts exhibited no cytotoxic activity against J774 and THP-1 cell lines (IC_{50}

> 500 µg/mL) and therefore showed excellent selectivity indexes (SI > 5). PCA score plots revealed good correlations between chemical profile and biological activity.

PA31 Alpha amylase inhibitory and Antioxidant activity of *Padina boergesenii* (*Allander and Kraft*) from Persian Gulf

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Among marine organisms, algae are a large and diverse group of organisms from which a wide range of secondary metabolites have been isolated. A number of these compounds possess biological activity such as toxicity, antibacterial, antifungal, antiviral, anti-tumor, anti diabetic and other specific activities (1 – 3). *Padina boergesenii* (*Allander and Kraft*) is the most abundant algae distributed in the Persian Gulf. In this study the Ferric Reducing Ability of Plasma (FRAP) activity and hypoglycemic effect was determined. methanol extract from *P. boergesenii* showed a considerable antioxidant effect 145.5 µ mol/lit (concentration of extract used = 20 mg/ml)and also higher inhibitory activity than the positive control against α-amylase. The IC_{50} value of *P. boergesenii* methanolic extract againstα-amylase was 0.29 mg/ml. (Concentrations: 0.05, 0.1, 0.25, 0.5 mg/ml). Acarbose (IC 50 = 0.53 mg/ml) was used as a positive control. Antioxidant and antidiabetic activity might be due to the presence of carotenoid, sterol, fucoxanthin, active phenolic compounds, and monoterpenes in the extracts. **References:** [1] Cannell R. J. P. Algae as a source of biologically active products. *Pest. Sci.* **39**: 147 – 153 (2006). [2] Rosa S.D., Kamenarska Z., Bankova V. Stefanov K., Dimitrova-Konaklieva S., Nadjenski H., Tzevtkova I. and Popov S. Chemical composition and biological activities of the Black Sea algae *Polysiphonia denudate* (Dillw.) kutz. and *P. denudate f. fragilis* (sperk) woronich. *Z. Naturforsch.* **56c**: 1008 – 1014 (2001). [3] Mazumder S., Ghosal P.K., and Pujol C.A. Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticata* (Gracilariaceae, Rhodophyta). *Int. J. Biol. Macromol.* **31**: 87 – 95 (2002).

PA32 Neglected parasitic diseases and therapy with medicinal plants used by people from the neighboring basins Platinum and Amazon

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Disease therapy using the flora has been maintained throughout the history of humanity. Substances in plants may be useful to treat neglected parasitic diseases (NPD). Chagas disease, endemic in the Amazon, is an NPD. The bugs *Triatoma infestans* and *T. sordida* are the vectors of the parasite, *Trypanosoma cruzi.* Schistosomiasis is another NPD caused by the helminth *Schistosoma mansoni*. The adult parasite is in the body of the patient and sporocysts in snail (*Biomphalaria*). It is endemic in parts of southeastern and northeastern Brazil. Leishmaniasis is another NPD caused by protozoa (*Leishmania* spp.). Vectors are mosquitoes (*Lutzomia* spp.) living in natural habitats but also in some cities, affecting dogs and man. The vector contracts the protozoan while sucking infected blood and infects new victims by further bites. The three diseases occur mostly in areas with poor health infrastructure, leading many patients to death. This study reveals plants used to treat these NPD by people from the neighboring region of the Amazon Basin (A), Platinum Basin (P); range Transition AP (T). We visited 21 counties (A = 4, T = 3, P = 14) of southwestern Mato Grosso (Brazil, 2005) to interview 63 informants with expertise in NPD (A = 12, T = 9, P = 42). They were asked which herbs were most important and served to treat these diseases. Chagas was more distressing in P (Cáceres) and T (VS Domingos); Schistosomiasis in the T (P. Lacerda, VS Domingos) and Leishmaniasis in A (Conq. Oeste) and P (Curvelândia, Indiavaí, P. Espiridião). Informants showed 2 plants used against Chagas *(Vochysia rufa, Echinodorus macrophyllus),* 2 plants against Schistosomiasis *(Commelina* spp., *Trianosperma trilobata)* and 5 plants against Leishmaniasis *(Xanthosoma violaceum, Symphytum officinale, Pterodon emarginatus, Atropa belladonna*, *Stryphnodendron adstringens).* Only few scienific studies are available online in April 2013 linking these plants to the three diseases. Further research in this area is suggested.

PA33 Plants used to treat malaria by people from the neighboring basins Platinum and Brazilian Amazon

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Medicinal plants help in the therapy of neglected diseases such as malaria, affecting mostly poor people. The mosquito vectors multiply in the tropics in margins of lakes, rivers and between vegetation. Malaria challenges the effectiveness of the public health service. Prevention is accomplished through the application of measures of biological, physical and chemical control. The use of bioactive plants can help in the fight against mosquitoes and treat disease. This study presents plants used to treat malaria by people from the neighboring basins Amazon (A), Platinum (P) and transition zone (AP = T). Municipalities were visited (21) in the southwest of Mato Grosso (Brazil, 2005) to collect data through interviews with informants (63) with expertise in the topic as indicated by the local community. Respondents were asked what were the most important medicinal plants and which purpose they served. Among the target municipalities (A: 4, PA: 3, P: 14), malaria was cited as a distressing disease in 8 of them (A:Comodoro, Vila BS Trindade; T: Vale S Domingos; P:Figueirópolis-Oeste, Glória-Oeste, Lambari-Oeste, Porto Estrela). Ten informants (A: 4, AP: 1, P: 5) indicated the use of 13 plant species (A: 5, AP: 2, P: 7) for malaria: "Caçau" (*Aristolochia triangularis*), "Cipó-mil-homens" (*Aristolochia esperanzae*); "Cordão-de-frade" (*Leonotis nepetifolia*), "Macaé" (*Leonurus japonicus*), "Cordão-São-Francisco" (*Leucas martinicensis*), "Esponja" (*Luffa acutangula*), "Fedegoso" (*Senna occidentalis*), "Fruta-galinha" (*Acnistus arborescens*), "Negramina" (*Siparuna guianensis*), "Melão-São Caetano" (*Momordica charantia*), "Erva d'anta" (*Psychotria laciniata*), "Erva d'cágado" (*Corchorus hirtus*), "Vassourinha" (*Scoparia dulcis*). Plants with more and fewer studies associated with malaria, available online in April 2013, are *M. charantia* and *P. laciniata*. We suggest further studies on these species' usefulness against malaria.

PA34 Plants used to treat Dengue by people from the neighboring platinum and Amazon basins, southwestern portion of Mato Grosso, Brazil

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Plants with bioactive compounds can help in public health actions. Dengue fever is a neglected disease. It affects all social classes, ages but especially the poorest. Hematophagous mosquitoes (dengue vectors) proliferate in accumulated waters. Dengue can be hemorrhagic and in this case it is serious and can lead to death. It is a challenge to public health, especially in the tropics. Peaks of maximum occurrence are in the rainy season, when emergency rooms are often congested. The fight begins eliminating standing water, but this should be associated with other measures such as protection from mosquitoes, by use of screens, nets, fans, repellents; application of mosquitocides, larvicides in infested areas. Some plants, like *Anacardium* spp. can help, through their bioactive substances, to combat the mosquito vector; still other plants can help in caring for patients. This study aims to present plants used to treat dengue by people who inhabit the borderland basins Platinum (P) and Amazon (A). In 2005, municipalities were visited (P: 14, PA: 3, A: 4) in southwestern Mato Grosso, Brazil for data collection, through interviews, made with informants (63) considered the most knowledgeable in the subject and indicated by the local community. People wanted to know which were the most important medicinal plants and for what purpose they are used. Dengue has been mentioned as painful in three counties (P: Cáceres, Porto Estrela, Araputanga). To treat dengue, informants (P = 5, A = 0) indicated the use of five plant species: "Acerola" (*Malpighia glabra* L.), "Esponja vegetal" (*Luffa acutangula* (L.)Roxb.), "Manga" (*Mangifera indica* L.), "Melão-de-São-Caetano" (*Momordica*

charantia L.), "Canela" (*Cinnamomum zeylanicum* Blume). Species for which more or less scientific studies associated with dengue were available online in April 2013, are, respectively: *C. zeylanicum* and *M. charantia*; *M. glabra* and *L. acutangula*. Further studies on these plants usefulness for dengue are suggested.

PA35 Plants used to treat two mycobacterial diseases neglected by people of southwestern Brazilian Amazon Basin and adjacent regions

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Herbal preparations may help controlling neglected mycobacterial diseases **(NMD). NMD** include Leprosy -*Ml (Mycobacterium leprae)* and tuberculosis -*Mt (M. tuberculosis). Ml* is a very infective disease, that manifests itself in the skin and nerves. In 2007, new cases of *Ml* (n°/10^5 inhab.) in Mato Grosso (100.2) were the largest in Brazil (21.9), and Amazon contributes 40% of cases. In the Americas, Brazil has most cases (94%), and in the world, it is the 2nd, after India. *Mt* is also a serious disease, but it develops mostly in more elderly and weak, often as a co-infection. The lungs and other organs are affected and the disease may ulimately lead to death. In Brazil, between 2006 – 2010, the Amazon had the 2nd highest rate of *Mt* (67.59 new cases/10^5 inhab.), the 3 rd highest mortality and the highest mortality and lethality in cases of co-infection (*Mt*/HIV). Both **NMD** affect mostly those who live in dirty and clustered environment. Current therapy, if applied early and well exercised, is effective. Persons at risk seek options to take care of their health, including plants. We questioned people in 26 counties bordering the Amazon Basin (A:5); Platinum (P:16) and Transition AP (T:5) about plants used to treat these **NMD**. The data came from several studies (PLAMED; PLAMEDIA; RAIZEIROS; PLAMUN-MT) in response to the question which medicinal plants are the most important to treat disease. Leprosy *(Ml)* is presented as distressing disease in 9 counties (A:3,P:3,T:3) and to treat it eight plants area used: *Rosa alba, Jacaranda cuspidifolia, Operculina hamiltonii, Echinodorus macrophyllus, Croton urucurana, Solidago microglossa, Mentha piperita, Baccharis dracunculifolia.* Tuberculosis *(Mt)* was distressing in 3 counties (A:1,T:1,P:1) and *Copaifera langsdorffii, Equisetum arvense* and *Curcuma longa* are used to treat it. For each disease *(Ml,Mt)* the plants with most and least scientific studies available (online-free access, Apr. 2013), are the first and the last species mentioned.

PA36 In silico prediction and experimental evaluation of furanoheliangolides as potent antitrypanosomal agents

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Continuing our studies on QSAR for the activity of sesquiterpene lactones (STL) against *Trypanosoma brucei rhodesiense (Tbr)* [1], we have extended the set of available IC_{50} data to 69, and generated a new QSAR model [2] in a similar way as reported previously. Descriptors were calculated from optimized 3D structure models. Compounds were divided into a training- and a test set (46/23). The training set descriptor matrix was submitted to genetic algorithm-based variable selection/multiple linear regression (GA-MLR). The best model regarding internal and external predictions (R^2= 0.75, Q^2= 0.65, P^2(test set)= 0.35) was employed to predict the activities for a database of 1700 STL structures. Quite notably, among the 71 compounds predicted to be highly active ($IC_{50} \leq 0.1\,\mu M$), 15 were STL of the furanoheliangolide (FH) subclass, which had not been tested for anti-*Tbr* activity before. Hence, four FH (**1**-**4**) were tested *in vitro*.

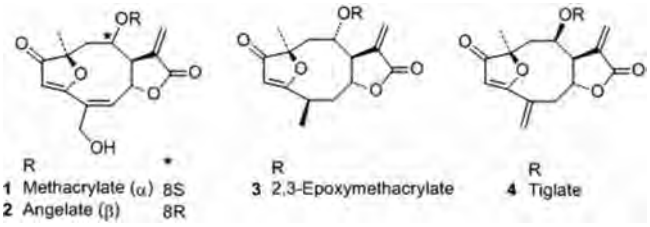

Fig. 1

Goyazensolide (**1**), budlein A (**2**), 15-deoxy-4,5-dihydro-2',3'-epoxygoyazensolide (**3**) and 4,15-isoatriplicolide tiglate (**4**) displayed IC_{50} values of 0.07, 0.07, 0.20 and 0.02 μM, respectively. **4** is the most active STL against *Tbr* found up to present. The lower activity of **3** is due to the absence of the α,β,γ,δ-unsaturated ketone moiety. In agreement with our earlier observations [1], the presence of a second Michael acceptor in addition to the α-methylene-γ-lactone group is required for high anti-*Tbr* activity. The experimental results confirm the usefulness of our QSAR model to predict the activity of untested STL. FH represent a new class of very interesting lead compounds against *Tbr* which deserve further investigations. **References:** [1] Schmidt TJ et al. Molecules, 14, 2062 – 76 (2009) [2] For all calculations: Molecular Operating Environment 2011.10 (MOE), Chemical Computing Group, Montreal, Canada, http://www.chemcomp.com/ This work is part of the activities of ResNetNPND: www.uni-muenster.de/ResNetNPND/ Support of CCG, Montreal, is gratefully acknowledged.

PA37 Antifungal compounds from *Vitis vinifera* canes against major grapevine fungal pathogens

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Worldwide the vast majority of grapevine areas are planted with *Vitis vinifera* cultivars that are all susceptible to various fungal diseases such as downy (*Plasmopara viticola*) and powdery (*Erysiphe necator*) mildews and grey mould (*Botrytis cinerea*). Since *V. vinifera* canes represent an unexploited agronomical waste material, their biological activity against the major grapevine pathogens and their chemical content were investigated in order to determine if extracts or compounds could be potentially used in a sustainable manner to protect grapevines. Methanolic and ethanolic crude extracts of *Vitis vinifera* canes exhibited significant antifungal activity against the three major fungal pathogens of grapevine: *P. viticola, E. necator* and *B. cinerea*. The active extract was analysed by LC-PDA-ESI-MS and some compounds were dereplicated. SPE-fractionation combined with the different antifungal assays enabled the localisation of the active zone in the HPLC chromatogram. Efficient targeted isolation using medium pressure liquid chromatography (MPLC-UV) afforded in one step six pure constituents (Ampelopsin A, Hopeaphenol, *trans*-resveratrol, Ampelopsin H, ε-viniferin and *E*-vitisin B). The structures of the isolated compounds were elucidated by 2D NMR and HR-MS. Six identified compounds presented interesting antifungal activities against *P. viticola.* **Acknowledgements:** Réflexion scientifique et technique des Neuf, Bordeaux, France.

PA38 Cytotoxicity of the essential oils from Tajikistan plants against *HeLa* cells

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The study was aimed to evaluate the anticancer activity of the essential oils from selected aromatic plants growing wild in Tajikistan against *HeLa* cells. The essential oils of *Anethum graveolens (AG)*, *Foeniculum vulgare* (FV), *Galagania fragrantissima* (GF) (Apiaceae), *Achillea filipendulina* (AF), *Artemisia absinthium* (AAb), *Artemisia annua* (AAn), *Artemisia rutifolia* (AR), *Artemisia scoparia* (AS) (Asteraceae), *Hypericum perforatum* (HP) (Clusiaceae) and *Hyssopus seravschanicus* (HS), *Melissa officinalis* (MO), *Mentha longifolia* (ML), *Origanum tyttanthum* (OT), *Salvia sclarea* (SS), and *Ziziphora clinopodioides* (ZC) (Lamiaceae) have been

obtained from fresh plant material by hydrodistillation. The cytotoxicity of the essential oils was determined using the MTT assay. IC_{50} values of cytotoxicity ranged between 0.09 and 0.96 mg/ml (Table 1). Their activity was in descending order: AG > AAb > AAn > FV > GF > HP > MO > OT > ML > ZC > SS > AS > AR > AF > HS. The essential oil of *Anethum graveolens L.* exhibited the strongest effect with an IC_{50} of 0.09 mg ml^{-1}, while the weakest effect was noted for the essential oil of *Hyssopus seravschanicus* (IC_{50} 0.96 mg ml^{-1}).

Tab. 1: Cytotoxicity of essential oils from Tajikistan plants against *HeLa* cells

Plants		IC_{50}, mg/ml	Plants		IC_{50}, mg/ml
Species	Family		Species	Family	
Anethum graveolens L.	Apiaceae	0.09	*Hypericum perforatum* L.	Clusiaceae	0.22
Foeniculum vulgare Mill.	Apiaceae	0.12	*Hyssopus seravschanicus* Pazij	Lamiaceae	0.96
Galagania fragrantissima Lipsky	Apiaceae	0.21	*Melissa officinalis* L.	Lamiaceae	0.29
Achillea filipendulina Lam.	Asteraceae	0.92	*Mentha longifolia* (L.) Huds.	Lamiaceae	0.33
Artemisia absinthium L.	Asteraceae	0.10	*Origanum tyttanthum* Gontsch.	Lamiaceae	0.32
Artemisia annua L.	Asteraceae	0.11	*Salvia sclarea* L.	Lamiaceae	0.51
Artemisia rutifolia Stephan ex Spreng.	Asteraceae	0.91	*Ziziphora clinopodioides* Lam.	Lamiaceae	0.42
Artemisia scoparia Waldst. & Kit.	Asteraceae	0.52			

PA39 Natural-product-based drug design against *Leishmania*: Addition of thiols to o-quinone methide for the synthesis of new 2-Hydroxy-3-phenylsulfanylmethyl-[1,4]-naphthoquinones

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Natural naphthoquinones as lapachol have been used as scaffold for the synthesis of antiparasitic compounds. Visceral Leishmaniasis (VL) is a fatal neglected disease caused by the protozoan *Leishmania*; widespread in 68 countries, VL victimizes 60,000 people per year. Considering the limited and highly toxic therapy and the increasing number of resistance, the need for novel drugs is imperative. The objective of this work was the development of a reaction involving three components for the preparation of phenylsulfanylmethyl-[1,4]-naphthoquinone, via o-quinone methide, structurally related with lapachol and its evaluation against *Leishmania infantum.* Thirty six new phenylsulfanylmethyl-[1,4]-naphthoquinones were obtained by a three component reaction of lawsone, formaldehyde and thiols adequately substituted. These reactions explored the *in situ* generation of o-quinone methides (o-QM) via the Knoevenagel condensation. The 50% Inhibitory Concentration (IC_{50}) of compounds was *in vitro* determined against *Leishmania infantum* promastigotes (MTT assay) and intracellular amastigotes (light microscopy). The cytotoxicity was determined in NCTC cells by the MTT assay. Among the thirty six synthetic naphthoquinones, 75% (27 compounds) showed activity against *L. infantum* promastigotes, with IC_{50} values in a range between 8 to 188 μM. Intracellular amastigotes were also susceptible to compounds; 38% (14 compounds) showed activity with IC_{50} values in a range between 12 to 57 μM. Considering the selectivity index (S.I.), compounds (1) and (2) were the most promising (S.I.> 8).

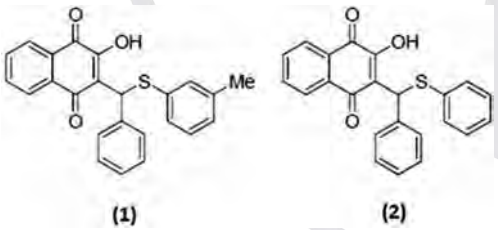

Fig. 1

The introduction of apolar groups to the 1,4-naphtoquinone rendered higher selectivity towards the intracellular amastigotes. Although the activity level is still rather moderate, some of these compounds may be interesting starting points for further optimization. Natural-product-based drug design has been a promising tool for the study of novel drug candidates. Supported by FAPESP, FAPERJ

PA40 Comparison of the chemical composition, antibacterial and antioxidant properties of Mango peal at three different stages of maturation

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Introduction: The mango fruit is among the most consumed fruits in the world and it has several traditional reported uses. Chemical components like flavonoids, vitamins, proteins have been isolated and biological activities such as antioxidant; antibacterial and antidiabetic properties are reported. The fruit has been largely studied, but little attention has been paid to the beneficial effect of the peal **Objective**: The aim of this study was to determine and compare the chemical composition of peal collected from mango fruits at three different stages of maturation: unripe, mid-mature and mature fruits; and to determine their antibacterial properties on *Salmonella typhi*, *Escherichia coli* and *Staphyloccocus aureus,* three microorganisms responsible of the most encountered infectious diseases in Mali. **Material**: Peals collected from unripe, mid-mature and mature mango fruits have been air-dried and pulverized. Antibacterial test was performed with clinical strains of *Staphylococcus aureus* and *Escherichia* and the reference strain 451 of *Salmonella typhi*. **Methods**: Tests for chemical analysis were carried out on peal powder using conventional test reagents that give specific color with a specific chemical group. Monosaccharide composition was determined by gas chromatography analysis after methanolysis. The antibacterial activities were evaluated using the disk diffusion technique and the antioxidant test was performed by applying as solution of DPPH on TLC plate. **Results and Discussion:** Results of the chemical investigation showed that the mango peal content of anthocyanins carotenoids, sterols and triterpenes and amounts of monosaccharides increase with maturation; glucose and glucuronic acid were the most abundant monosaccharides. Aqueous extracts of mid-mature mango fruit peal showed a moderate inhibition of *S. aureus* at a dose of 200 μg while mature and unripe fruits peals extracts showed no activity. A high DPPH inhibition activity was seen with all samples.

PA41 Green Tea halts progression of cardiac involvement in senile systemic amyloidosis

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Amyloidosis labels a group of diseases that is characterized by aggregation of beta-sheated fibrillar proteins and deposit in the extracellular space. In general amyloid can be found in almost all organs, but senile systemic amyloidosis (SSA) is characterized by sole cardiac involvement resulting in organ dysfunction and finally organ failure. There is no causative therapy for patients with SSA and standard heart failure medication is not able to slow progression of the disease. Recent reports indicate the potential of epigallocatechin-3-gallate (EGCG), the most abundant catechin in green tea (GT), to inhibit fibril formation from several amyloidogenic proteins in in vitro experiments. We sought to investigate the effect of GT on patients with SSA. 15 male Patients (73.2 ± 7.8 kg) with histological proven SSA underwent echocardiography (EC), blood analysis and cardiac magnetic resonance imaging (CMR) before and after daily consumption of capsules containing 1200 mg of green tea extract with an EGCG content of at least 50% (extraction solvent: ethyl acetate/ethanol/water) for 12 months. After 12 months of GT consumption a significant decrease of LV myocardial mass (-15.4 ± 32.9 g;-7 ± 15 %;$p < 0.05$) was observed by CMR. LV ejection fraction remained unchanged (+1.7 ± 32.4%;$p = 0.35$). By EC no significant increase of left ventricular (LV) wall thickness (+1.8 ± 0.7 %;$p = 0.87$) or decrease of mitral annular plane (MAP) velocity (-10.1 ± 19.1 %;$p = 0.39$) were observed, but MAP systolic excursion decreased by 16.2 ± 21.6% ($p < 0.05$). Renal function remained unchanged (-4.1 ± 25.6%; $p = 0.51$). Moreover, a significant reduction of overall cholesterine was observed (-8.7 ± 35.5%; $p < 0.05$). No serious adverse events were reported by any of the participants. Due to reduction of LV mass observed in the present

study an inhibitory effect of GT on amyloid fibrils can be assumed during 12 months of treatment. Long-term effects and impact on the overall survival need to be confirmed in a larger placebo-controlled study.

B. *In vivo* phytopharmacology

PB1 Non-antibiotic herbal therapy of uncomplicated lower urinary tract infection in women – a pilot study

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Objectives: Despite of increasing prevalence of bacterial resistance to antibiotics and possible adverse drug reactions (ADRs), uncomplicated lower urinary tract infections (uUTIs) are usually treated with antibiotics. This pilot study had the objective to assess safety and impact of a non-antibiotic therapy with Canephron®N, an herbal medicinal product. **Methods:** This open-label, non-randomized, interventional trial was performed in 9 Ukrainian sites. 125 women aged 20 to 65 years suffering from an acute symptomatic episode of uUTI with a sum score of at least 6 for the symptoms dysuria, frequency, urgency (each rated 0 – 4) were enroled. Patients were treated with 3 x 2 tablets Canephron®N for 7 days. Symptom assessment was performed on day 0 (d0), day 7 (d7) and day 37 (d37) by the investigators. The primary endpoint was incidence of ADRs during treatment. Secondary endpoints were clinical responders (all 3 symptoms scored as absent (0) or mild (1)) and symptom severity on d7 and d37, patients requiring antibiotic treatment until d7, duration of uUTI symptoms and patients with recurrence on d37. **Results:** None of the reported 19 adverse events (AEs) was considered as drug related (ADR = 0%; no serious AEs). At the end of treatment, 71.2% of patients met the definition of a responder (85.6% at d37). The sum score decreased from 7.3 at d0 to 1.9 (d7) and 0.7 (d37), respectively. The mean values (d0/d7/d37) of the symptoms were: dysuria 2.5/0.6/0.2; frequency 2.7/0.9/0.3; urgency 2.1/0.5/0.2 (all changes from baseline $p < 0.001$). Three patients (2.4%) required antibiotics between d0 and d7. On average, the time to symptom resolution in days was: 5.1 (frequency), 3.5 (dysuria), 2.9 (urgency). None of the responder had recurrence on d37. **Conclusions:** The results of this study substantiate our hypothesis that Canephron®N may be an effective and well tolerated alternative treatment of uUTI, reducing the use of antibiotics. Further controlled clinical trials are considered.

PB2 Proanthocyanidin-enriched extract from *Rumex acetosa* L. as a prophylactic agent against *Porphyromonas gingivalis*: a clinical pilot trial

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Aims: Periodontitis is a biofilm depended oral infection. It leads to inflammatory destruction of periodontal tissue and can also affect systemically the host's immune response. *Porphyromonas gingivalis* (*P.g.*) is one of the major pathogens associated with the onset and progression of periodontitis. The bacterial adhesion to oral mucosa cells is primarily essential for the tissue invasion and following breakdown. Previous *in vitro* studies have shown that a proanthocyanidin-enriched extract from *Rumex acetosa* L. inhibits the adhaesion of *P.g.* and acts in a cytoprotective manner. Therefore the aim of this controlled, randomized and double blinded study was to evaluate these effects in humans. **Material and Methods:** 35 *P.g.* positive, but periodontally and generally healthy patients received a supragingival debridement. Afterwards they were randomly assigned to the test or control group and were instructed to rinse 3 times per day with either a *Rumex acetosa* extract (0.8% w/w) containing mouth wash or placebo for 7 days. Plaque samples were taken at different visits (screening, baseline, 2, 4, 7 and 14 days after baseline) and *P.g.* was identified and quantified by real-time polymerase chain reaction. Additionally clinical parameters as plaque- and bleeding index were recorded. **Results:** Preliminary microbiological results did not show any significant differences, but the median of the *P.g.* prevalence showed a tendential greater increase in the test group. A significant reduction of the gingival bleeding index was monitored in the test group at day 14 ($p = 0.003$) compared to the baseline values, this was not detected in the control group ($p = 0.074$). **Conclusion:** This study indicates that the usage of a *Rumex acetosa* extract in combination with professional tooth cleaning results in a favorable anti-inflammatory outcome in *P.g.* positive patients. Further investigations have to be made regarding this cytoprotective effect and the heterogenic outcome of the *P.g.* prevalence.

PB3 Possible therapeutic role of *Curcuma longa* extract against colon cancer experimental model

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Objective: This study aimed to investigate the role of *Curcuma longa* (Turmeric) methanolic extract against colon cancer-induced in rats. Materials and **Methods:** Forty male rats were classified into 5 groups. Group (1) was control. Groups from (2) to (5) were intrarectally injected with N-methylnitrosourea for induction of colon cancer then group (2) was left untreated (cancer group); group (3) was treated intraperitoneally with 5-fluorouracil, while, groups (4 and 5) were treated orally with 437.5 mg/kg and 875 mg/kg of turmeric extract respectively. Histological investigation of colon tissue was done. Colon β-catenin and K-ras gene expression was detected by RT-PCR. Immunohistochemical technique was used for estimation of colon COX-2 and survivin expression. Plasma TGF-β and Bcl-2 and serum CEA and CCSA-4 levels were assayed using ELISA procedure. **Results:** Histopathological investigation of colon tissue sections in cancer group showed dysplasia and anaplasis in the lining epithelial cells of the glandular structure. While, treatment with 5-fluorouracil or turmeric extract showed marked improvement in the histological structure of colon tissue. Cancer group showed significant increase in the expression level of β-catenin and K-ras genes. While, all treated groups showed significant decrease in the expression levels of these genes. Colon cancer group showed significant increase in colon COX-2 and survivin expression. On the other hand, all treated groups exhibited marked decrease in COX-2 and survivin expression. Colon cancer group showed significant elevation in the studied biochemical markers. In the contrary, all treated groups showed significant reduction in these markers. Conclusion: It could be concluded that *Curcuma longa* methanolic extract, in the above mentioned doses, has a promising therapeutic role against colon cancer induced in rats as it has anti-inflammatory, antiproliferation and apoptotic effects.

PB4 Hypoglycemic effect of *Malmea depressa*, a plant used in the treatment of Type 2 Diabetes in Yucatan, Mexico

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Diabetes mellitus type 2 (DMT2), is characterized by tissue insulin resistance combined with a relative deficiency in insulin secretion. An individual may present primarily with insulin resistance or beta cell deficiencies, and these abnormalities can range from mild to severe. DMT2 is one of the most prevalent health problems in Mexico. As a result of the application of the Disease-Consensus Index, *Malmea depressa* R. E. Fries, (Annonaceae) (MD) was selected as the prominent specie traditionally used in a Mayan community to treat DMT2. The acute and chronic hypoglycemic effects were confirmed in neonatally streptozotocin induced diabetic rats (n5-STZ), thereafter was determined whether MD would reduce hepatic glucose production by targeting gluconeogenesis. The effect of the plant extracts on gluconeogenesis (in vivo), and the activity over Glucose-6-Phosphatase (in vitro) were examined. Furthermore, the phytochemical composition of the plant was analysed. From the pharmacological active fractions, two Phenylbutane derivatives (2-Hydroxy-3,4,5-trimethoxy-1-(2',4'-hydroxy-3'-dihydroxy) butyl-benzene and 2-Hydroxy-3,4,5-trimethoxy-1-(2',3',4'-hydroxy)butyl-benzene) as well as a phenylpropane derivative, 3-(3-hydroxy-2,4,5-trimethoxyphenyl) propane-1,2 diol, were isolated. To assess the degree of inhibition of glucose-6-phosphate hydrolysis, we plotted a dose-response curve; the ethanolic extract poses an IC50 of 267.62 (μg/ml). The results suggest that administration of MD can improve glycemic control by blocking hepatic glucose production, especially in the fasting

state. These data support its traditional use as an infusion consumed continually throughout the day.

PB5 Investigation of anti-ulcer activity of *Ficus bengalensis* Linn bark in laboratory animals

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Ficus bengalensis (Moraceae) is commonly known as a Banyan tree and Vada tree in Ayurveda. The plant is a large evergreen tree distributed almost all over the world and is used medicinally for treatment of different disorder. The present study was performed to evaluate the anti-ulcer activity of hydro-alcoholic extract of bark of *Ficus bengalensis* against ethanol-induced gastric mucosal injury in rats and pylorus ligation gastric secretion in rats. The freshly prepared hydro-alcoholic extract (Methanol70%+Water30%) was qualitatively tested for the presence of major phytochemical constituents which revealed the presence of flavonoids, saponins sugar and tannins. For evaluation of antiulcer activity five groups of adult wistar rats were orally pre-treated respectively with carboxy methyl cellulose (CMC) solution (ulcer control group), Omeprazole 20 mg/kg (reference group), and 100, 200 and 300 mg/kg extract in CMC solution (experimental groups), one hour before oral administration of absolute ethanol to generate gastric mucosal injury. Rats were sacrificed and the ulcer index, gastric volume, gastric pH, free acidity, total acidity of the gastric content was determined. Grossly, the ulcer control group exhibited severe mucosal injury, whereas pre-treatment extract exhibited significant protection of gastric mucosal injury in both the models. Histological studies revealed that ulcer control group exhibited severe damage of gastric mucosa, along with edema and leucocytes infiltration of submucosal layer compared to rats pre-treated with extract which showed gastric mucosal protection, reduction or absence of edema and leucocytes infiltration of submucosal layer. Acute toxicity study did not manifest any toxicological signs in rats. The present finding suggests that *F. bengalensis* bark extract promotes ulcer protection as ascertained großly and histologically compared to the ulcer control group which may be attributed to the presence of flavonoids or saponins.

PB6 The methanolic extract of *Ficus bengalensis* and its fraction induces antihepatotoxic activity *in vivo*: possible involvement of antioxidant action

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Ficus bengalensis (Moraceae) known as a Banyan tree Vada tree in Ayurveda. The plant is a large evergreen tree distributed almost all over the world and is used medicinally for treatment of different disorder. In the present study, the methanolic extract of bark *Ficus bengalensis* and its different fractions were evaluated for their hepatoprotective activity against CCl_4 induced hepatotoxicity. The methanolic extract was prepared and fractionated using the solvents of varying polarity like toluene, chloroform, ethyl acetate and n-butanol and tested for hepatoprotective activity in the rats. Extent of hepatic damage was assessed by levels of SGPT, SGOT, ALP, Total Bilirubin and Direct Bilirubin and histopathologic study of liver sections. Probable mechanism was investigated by carrying out free radical scavenging activity of extract and its fractions. There was a significant increase in the levels of serum GOT, GPT after administration of CCl_4 (0.7 ml/kg) to rats. Methanolic extract (100 mg/kg) and (250 mg/kg) and ethyl acetate fraction (50 mg/kg) produced significant reduction in the level of these enzymes.

Tab. 1: % decrease in levels of enzymes and bilirubin by methanolic extract of *Ficus bengalensis* and its ethyl acetate fraction as compared to CCl_4 treated control group

Extract/Fraction	Methanolic Extract	Methanolic Extract	Ethyl Acetate Fraction
Parameter	(100 mg/kg)	(250 mg/kg)	(50 mg/kg)
SGPT (%)	56.17	72.81	70.90
SGOT (%)	39.58	55.82	51.77
ALP (%)	43.59	54.86	39.64
Bilirubin (Total) (%)	33.50	33.50	61.00
Bilirubin (Direct) (%)	67.06	67.05	71.16

Methanolic extract and its ethyl acetate fraction also preserve the structural integrity of the hepatocellular membrane as revealed from histological studies and it also exhibited significant antioxidant activity in tested models. Thus *F. bengalensis* bark exhibited antihepatotoxic effect and it appears that the hepatoprotection offered by *F. bengalensis* may be related to its antioxidant activity.

PB7 Immunotoxicological safety of preparations obtained from cells of *Mucor* species

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The cell biomass of *Mucor racemosus* Fresen. (DSM 2845) and *M. mucedo* L.: Fr. (minus strain: DSM 4885 and plus strain DSM 4886) gained after fermentation is purified from culture medium components and then mechanically opened through a cell mill. After different purification steps, the water-soluble filtrate undergoes sterile filtration and is freeze-dried. The resulting lyophilisate is characterized by electrophoresis (SDS-PAGE), carbohydrate composition of polysaccharides and content of total proteins. The resulted starting material is named "*e volumine cellulae (lyophil., steril.)*" (evc) and potentised to homeopathic dilutions or triturations used for the homeopathic treatment of disorders of blood circulatory system (Mucor racemosus) [1] or some chronic inflammations and nervous disorders (Mucor mucedo) [1]. Possible immunotoxic effects after repeated oral and rectal, intradermal/dermal or subcutaneous application were tested in various guideline studies with GLP compliance in genetic defined mice and guinea pigs. These studies include general immunotoxicity, mitogenic effects of naive T-cells, proliferation of antigen-stimulated T-cells, delayed type hypersensitivity reactivity, antigen-specific antibody production, acute systemic anaphylaxis induction, and skin sensitisation studies. It can be concluded that Mucor racemosus evc (Mucokehl®, Vetokehl Muc®) and Mucor mucedo evc (Mucedokehl®) can be regarded as safe in potency D3, D4 and D5, respectively. The immunotoxicological safety data are valid only for the investigated fungi strains as well as for the specific, GMP controlled manufacturing process. **Reference:** [1] Package leaflet Mucokehl and Mucedokehl, Swissmedic-Arzneimittelinformation, www.swissmedicinfo.ch

PB8 *Copaifera langsdorffii* leaves extract and its isolated compounds display gastric antiulcer activity

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Gastric ulcer is a major cause of morbidity and mortality among the digestive tract diseases. *Copaifera langsdorffii* Desff., popularly known as "copaiba," is a large tree that grows in Brazil. The trunk oil resin is used in folk medicine for a variety of diseases, including urinary, lung and gastric disorders [1]. The aim of this work was to evaluate the gastroprotective activity of *C. langsdorffii* leaves using the induced ulcer chronic model by acetic acid [2]. For that, *Swiss* male mice (n = 6) was operated for exposing the stomach, and 0.02 mL (v/v) of 30% acetic acid solution was injected into the submucosal layer in the junction between the antrum and the fundus. After two days of recovery, animals were orally treated during seven days with 500 mg/kg of *C. langsdorffii* leaves extract, cimetidine (100 mg/kg) and saline solution. After that, animals were killed and the gastric lesions were analyzed *macroscopically* using EARP software image-*analysis*. For evaluating the isolated compounds, the experiment was performed using ethanol/HCl-induced ulcer [3]. *Swiss* male mice (n = 6) received 30 mg/kg of α-humulene (1), β-caryophyllene (2), caryophyllene oxide (3), kaurenoic acid (4), afzelin (5) and quercitrin (6). Omeprazole (30 mg/kg) and saline solution were used as controls. After one hour of treatment, animals received 0.2 mL of ethanol/HCl solution (60%/0.3 M) to induce lesions, and one hour later they were killed for analyses. In the chronic protocol, leaves extract (500 mg/kg) and cimetidine exhibited gastroprotective activities of 73% and 70%, respectively. Compounds 1, 2 and 3 displayed activities higher than 70% in comparison with omeprazole (78%). The observed gastroprotective activities might be associated, in part, to its antioxidant properties. **References:** [1] Gramosa N. V., et al. *Rec Prog Med Plants,* 235 – 260,

2010. [2] Takagi K, Okabe S, Saziki R. *J Pharmacol*, 19: 418, 1969. [3] Mizui T, Doteuchi M. *Jpn J Pharmacol*, 33:939 – 945, 1983.

PB9 Extract preparation from *Sideritis scardica* enhances memorizing skills of mice in Morris water maze

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The Greek mountain tea is well known in Europe, occurring mainly in Turkey, Bulgaria and Greece. The botanical species hereof is *Sideritis scardica* Griseb. Over centuries it was used as relaxing tea (so called Shepard's tea or Mursalski tea). Actual *in vitro* investigations showed influence on reuptake-inhibition of neurological transmitters by *Sideritis scardica* extracts. Due to this, investigations were performed about in-vivo effects of Sideritis extracts relating to mental CNS disorders (Alzheimer's disease) and cognition. As an established test model for cognition and spatial memory, we used the Morris water maze (MWM). Beginning with this model 30 years ago, healthy mice were tested treatment group vs. non-treatment group. Newest investigations demonstrate that a within-subject comparison approach is both valid and effective in reducing variability. Today, transgenic AD mice are available which allow testing of active substances against AD. Therefore, we introduced a *Ginkgo biloba* extract as positive control which is used as off-label cognition enhancer in elderly and AD patients. Following four groups (n = 6) were tested:

1. transgenic AD mice (Alzheimer's disease model in C57BL/6 background, untreated as control 1);
2. healthy background control mice (C57BL/6, untreated as control 2);
3. transgenic AD mice treated with *Sideritis scardica* extract;
4. transgenic AD mice treated with *Ginkgo biloba* extract (acc. Ph.Eur.)

Healthy control group (2) showed reduced latencies (-20%) as compared to the transgenic group (1, AD control), which confirms the suitability of (1) for our approach. The group (3) of *Sideritis scardica* treated AD mice showed significant lower latencies (-60% vs. control 1, and -40% vs. control 2, resp.), whereas the group of Ginkgo treated mice did not differ from any control 1 or 2. The behavioral testing results for the Sideritis group (3) correlate with the histopathological finding – reduction of total ß-Amyloid amount by 55% vs. control (1).

PB10 Anti-inflammatory activity of geraniin and aqueous extract of *Phyllanthus muellerianus* (Kuntze) Exell.

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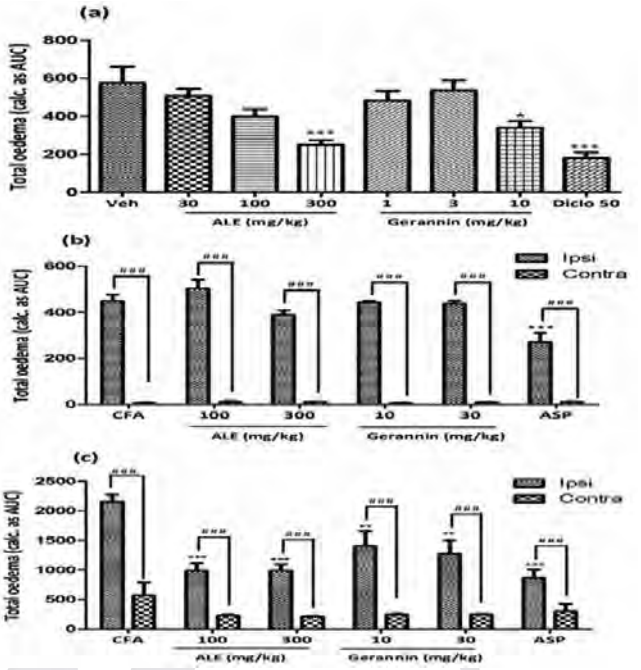
Phyllanthus muelllerianus (Kuntze) Exell. (Family Euphorbiaceae) is a tropical plant used for the management of menstrual disorders, fever and pains [1]. In this study, acute and chronic anti-inflammatory activity of aqueous leaf extract (ALE) of *P. muellerianus* and its isolate, geraniin, were investigated. Anti-inflammatory activity of ALE (30, 100, 300 mg/kg) and geraniin (1, 10, 30 mg/kg) were assessed in the carrageenan-induced paw oedema test [2] and adjuvant induced-arthritis (AIA) tests in male Sprague-Dawley rats (150 – 200 g) [3]. Diclofenac and Aspirin were used as reference drugs. ALE (at 300 mg/kg) and geraniin (at 10 mg/kg) showed significant (both $p < 0.001$) reduction in paw oedema in the carrageenan-induced paw oedema test in rats. The reference drug diclofenac also significantly reduced paw oedema at the dose of 50 mg/kg ($p < 0.001$). The anti-inflammatory activity of ALE and geraniin were dose-dependent (Fig 1). In the AIA, ALE (100 and 300 mg/kg) and geraniin (10 and 30 mg/kg) modified the time course curve and exhibited a dose-dependent oedema reduction in the ipsilateral paw. Aspirin (100 mg/kg) significantly ($p < 0.001$) inhibited polyarthritis oedema. Radiograph showed ALE- and geraniin- treated groups exhibited a dose-dependent inhibition of bone erosion and deformation. Geraniin showed a slightly better activity than ALE. Blood analysis of ALE and geraniin showed reduction in WBC, ESR and increase in RBC, hematocrit and hemoglobin levels which reveal the anti-inflammatory activity of treatment. Geraniin showed a better anti-inflammatory activity than ALE. ALE and geraniin exhibited *in vivo* anti-inflammatory activity.



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PB11 A multicentre open clinical trial to assess the tolerability and efficacy of Boldocynara®, a traditional herbal preparation for functional digestive disorders

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Functional digestive disorders lead the list of visits to gastroenterologists and affect up to 40% of the population, with only a minority seeking medical care for it. Boldocynara®, a proprietary combination product consisting of artichoke leaves, milk thistle fruits, dandelion herb and root, and boldo leaves, has been traditionally used to treat indigestion. In this study including 75 patients aged 18 to 71 years and suffering from functional digestive disorders at least twice weekly since at least 2 months, Boldocynara® applied in a newly developed solid form at a dosage of 2 x 1 tablet daily proved to be well tolerated and efficacious in reducing these complaints as evaluated via the SF-LDQ measuring frequency and severity of dyspepsia, heartburn, regurgitation and nausea and a Global questionnaire on fat intolerance, upper abdominal pain, epigastric discomfort, abdominal bloating, postprandial fullness, flatulence, abdominal cramps, constipation, diarrhoea and stool irregularities. All symptoms decreased significantly over the 6 weeks treatment period ($p < 0.0001$), reflected simultaneously by a significant improvement of all items assessed via the QoL-SF-12. The SF-LDQ total score decreased under therapy from 6.3 ± 2.9 to 1.4 ± 2.0 regarding frequency of dyspeptic symptoms and from 3.6 ± 3.8 to 0.7 ± 1.7 regarding their interference with normal activities, whilst the digestive symptoms evaluated with the global questionnaire total score decreased from 17.9 ± 6.4 to 4.9 ± 5.9 for their frequency and from 9.6 ± 9.7 to 1.2 ± 2.9 for their interference with normal activities. Tolerability was rated as very good or good by 89% of physicians and 91% of patients. The evaluation of the related laboratory parameters showed a high degree of safety. Boldocynara®, the tested traditional herbal blend, demonstrated in this study clearly good clinical value in terms of efficacy, safety and tolerability in the treatment of functional digestive disorders.

PB12 Protective effect of sub-fraction of methanolic extract of *Calotropis procera* latex in FCA induced arthritic rats

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The latex of the plant *Calotropis procera* has been reported to exhibit potent anti-inflammatory and anti-arthritic properties in various animal models. In the present study, the methanolic extract of latex of this plant was subjected to column chromatography and 11 fractions were separated using solvents of increasing polarity. In a preliminary screening, fraction No. 9 (F9) out of these 11 fractions was found to exhibit anti-inflammatory property and was further evaluated for its efficay in FCA induced arthritis model. Arthritis was induced by single intra-articular injection of 0.1 ml of 0.1% of Freund's Complete Adjuvant (FCA). The F9 (50 & 150 mg/kg) and diclofenac (5 mg/kg) were given orally 1 hr before injecting FCA and then daily for next 3 days. Parameters like joint diameter, functional disability (motility & dorsal flexion pain), levels of inflammatory markers (myeloperoxidase & nitric oxide), oxidative stress markers (superoxide dismutase & catalase) and tissue histology were measured. The efficacy of F9 was compared with standard anti-inflammatory drug diclofenac. The F9 showed a dose-dependent inhibition of joint inflammation and normalized the functional disability, levels of inflammatory markers and oxidative stress markers and the effect was comparable to that of diclofenac. The treatment of F9 was also found to maintain the tissue integrity in arthritic rats in comparison to that of arthritic control rats. Our results suggests that F9 has the therapeutic potential to be used in the treatment of various arthritic conditions. The charcterization (HPLC) and purification of the F9 has to be done in our future studies.

PB13 Effects of Silexan on EEG power spectrum of conscious freely moving rats

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Silexan is an essential oil of selected quality produced from the flowers of *Lavandula angustifolia*. Efficacy and safety of silexan in anxiety disorders has been demonstrated in controlled clinical trials[1] and open pilot studies. In Germany, it is approved for oral treatment of restlessness related to anxious mood. Goal of the present investigation was to test its effects in freely moving, day-night converted rats and to compare the results to synthetic drugs with similar clinical indications. Rats were implanted with four bipolar concentric electrodes into frontal cortex, hippocampus, striatum, and midbrain reticular formation. Field potentials were transmitted wirelessly, analyzed by fast Fourier transformation and split into six frequency ranges. Spectral power was averaged over 60 min periods. After a 45 min reference period silexan was orally administered and recording continued for further 5 h. Three doses (40, 80 and 120 mg/kg) were investigated during three consecutive days. No consistent effect was observed after administration of vehicle (1 ml/kg,0.2 %methylcellulose). In contrast, consistent increases of alpha1 and beta power as well as attenuations of spectral power in the delta and alpha2 range were seen after administration of silexan. Repetitive application for three days revealed very good reproducibility. Statistically highly significant spectral changes were seen at all dose levels during the first hour. Classification by comparison to reference drugs was achieved by use of discriminant analysis. In conclusion, silexan seems to possess calming down and mood elevating properties. Similar spectral patterns were induced by thioridazine, and metoprolol, drugs which are known for their relaxing and anxiolytic properties. Since comparable changes were also caused by compounds acting on the 5-HT_{1A} receptor, interference with serotonergic neurotransmission might be regarded as a mechanism of action. **Reference:** [1] Kasper et al, 2010 Wien Med Wochenschr. (21 – 22):547 – 56

PB14 Antiplatelet and antithrombotic effect of *Phyllostachys pubescens* leaves and Mume fructus combination

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Agent currently used for the treatment and prevention of thrombosis have a number of side effects. The aim of the present study was to investigate the ethanol extracts of *Phyllostachys pubescens* leaf, Mume fructus, and their combined preparations, on platelet aggregation *in vitro* and on rat arterio-venous (AV) shunt thrombosis *in vivo*. The 80% (*v*/*v*) ethanol extracts of *Phyllostachys pubescens* leaf (PL), Mume fructus (MF), and their combinations [2:1 (PM21), 1:1 (PM11), and 1:2 (PM12)] were evaluated in ADP-induced rat platelets *in vitro*. At 100 μg/ml, PM21 and PM11 inhibited *in vitro* ADP-induced aggregation by 44.0 ± 4.3% and 30.0 ± 3.2%, respectively, whereas PL, MF and PM12 weakly or scarcely inhibited ADP-induced aggregation by 3.9 ± 3.2%, 13.0 ± 2.7% and 5.2 ± 1.3%, respectively. The IC_{50} values of PM21 on ADP-, collagen- and thrombin-induced platelet aggregations were 135.6 ± 7.4 μg/ml, 142.7 ± 5.8 μg/ml and 186.5 ± 9.7 μg/ml, respectively. In an *in vivo* rat AV-shunt thrombosis model, the thrombus weight was significantly decreased after oral administration of a 400 mg/kg dose of PL (27.8 ± 3.0%, $p < 0.01$) or MF (35.2 ± 2.1%, $p < 0.01$), while that after administration of a 5 mg/kg of Riv (Rivarosaban), used as a positive control, were significantly decreased (61.7 ± 1.9%, $p < 0.001$), and with a good accord to the *in vitro* results, the combination of PL and MF in the ratio of 2:1, PM21 (60.9 ± 1.2%, $p < 0.001$), showed a superior anti-thrombotic effect to those of individual extracts. At dosages of 200, 100 and 50 mg/kg, PM21 dose-dependently decreased thrombosis weight (ED 50, 314 mg/kg). These results suggest that combinational preparations of PL and MF, and especially their 2:1 combination, can increase antiplatelet and anti-thrombosis effects more than PL and MF alone, offering evidence for a potential novel combination anti-thrombic therapy.

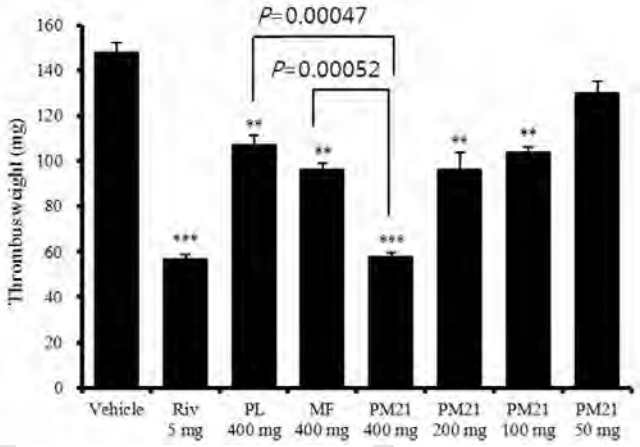

Fig. 1

PB15 Phytochemical Analysis and Antihepatotoxic activity of *Desmodium adscendens* decoction against chemically-induced liver damage

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Desmodium adscendens is a herbaceous plant occurring in Africa and South America. A decoction from leaves and stems is used in traditional medicine for various indications including its hepatoprotective effect. In the present study the protective and curative effect of *D. adscendens* decoction against chemically-induced liver damage in rats has been evaluated. The phytochemical composition of the decoction was also investigated. The protective effects against D-galactosamine-induced and ethanol-induced liver damage of a decoction of *D. adscendens*, quantified on its main constituent D-pinitol, was investigated in rats. Enzyme

levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), which are among the usual biomarkers for liver damage, were determined in serum samples. In addition, the curative effect of pure D-pinitol and *D. adscendens* against chronic D-galactosamine-induced and acute acetaminophen-induced hepatotoxicity in rats was studied. Silymarin was used as positive control. The results showed that *D. adscendens* decoction had a protective effect in rats against liver damage induced by D-galactosamine and ethanol, and this effect was at least in part due to the presence of D-pinitol. However, no curative effect of the decoction or D-pinitol on liver damage induced by the tested chemicals could be demonstrated[1]. The phytochemical content of the decoction was further investigated using flash chromatography and LC-SPE-NMR. Apart from D-pinitol, it was found to be rich in flavonoids which were mainly vitexin and its derivatives such as vitexin-2''-xyloside and isovitexin. These results justify at least in part the antihepatotoxic use of *D. adscendens* decoctions. **References:** [1] J Ethnopharmacol. 2013, 146, 250.

PB16 Evaluation Of Writhing And Paw Edema In Swiss Male Mice Of Sesquiterpene Lactones Fraction Obtained From *Artemisia annua* L.

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Introduction: *Artemisia annua* L. is known for antimalarial properties. The artemisinin production process generates high yields of sesquiterpene lactones fraction (1,72% artemisinin) by-products. In order to enable to access the pharmacological potency of the fraction an antiinflamatory experimental model was evaluated on writhing and paw edema assay induced by carrageenan in Swiss male mice. **Methods:** In both tests groups of Swiss mice (n = 6) were treated i.p with vehicle (10 ml/kg) and sesquiterpene lactones sample using 100, 300, and 500 mg/kg (i. p.) doses. Writhings were induced by an i. p. injection of 0.8% acetic acid solution (10 ml/kg), 30 min after treatment. After this injection, the numbers of writhings (abdominal constrictions) were cumulatively counted over 15 min, for nociception evaluation. For assessment of edema, 25 µL of a 3% solution carrageenan were injected subcutaneously into the plantar region of the left hind paw. Paw measurements (plethysmometer) were taken at 2, 4, 6 and 24hours. **Results:** Values are expressed as mean ± standard error. Data were analyzed by analysis for variance (ANOVA) and Tukey's test. In the writhing test values obtained were G1 (control) 48 ± 5,2; G2 (500 mg/kg) 9,5 ± 3,23; G3 (300 mg/kg) 31,5 ± 3,5; G4 (100 mg/kg) 48,5 ± 7,58). P < 0,05 G1 vs. G2, G2 vs. G3, G2 vs. G4. Paw edema induced by carrageenan are shown in the graph (Figure 1)

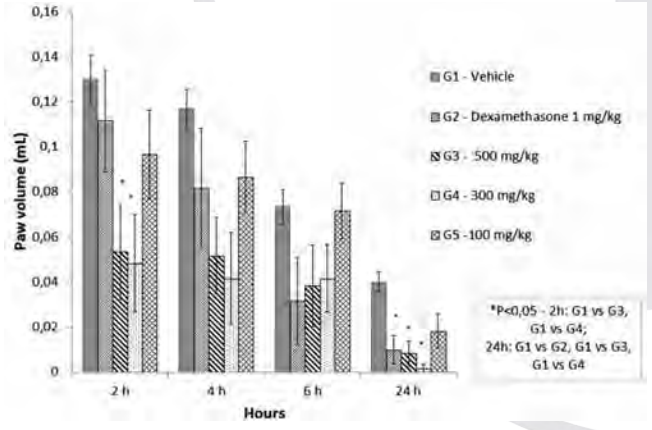

Fig. 1: Evaluation of different doses of sesquiterperne lactones fraction on inflammatory response post-carrageenan injection

Conclusions: In the writhing test the 500 mg/kg dose sesquiterpene lactones fraction proved to be the most effective in reducing abdominal constrictions, possibly by a nociceptive mechanisms. In the assessment of paw edema after 2 hours at 500 and 300 mg/kg doses proved to be more effective than the positive control, and this profile was maintained for up to 4 hours, suggesting an anti-inflammatory property of the sample. Further tests are needed to confirm these properties.

PB17 Lesion Inhibition Index of *Pterodon pubescens* Benth. Crude Extracts Evaluated On Ethanol Induced Ulcer Models In Rats

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Inflammation is a natural response to tissue infection or injury. Nevertheless, chronic inflammation has an adverse effect on the body, such as function loss of the affected tissue and thus decreases life quality of individuals. Glucocorticoids and NSAIDs are nowadays the main family of drugs used to treat inflammation although they frequently expose the gastric mucosa leading to gastric ulcer development and other side effects. Previous studies have demonstrated the anti-inflammatory activity of vouacapans, diterpene furans isolated from *P. pubescens* Benth species. With the intention of investigating possible side effects produced by this species this study had the aim to evaluate the antiulcerogenic activity of crude diclorometh ane (Pp) and aqueous (Ppa) extracts on gastric ulcer model induced by ethanol. The antiulcerogenic effect of Ppa and Pp extract of *P. pubescens* were evaluated in ethanol induced gastric ulcer model in Wistar rats. The extracts were administered orally at three different doses of 30, 100 and 300 mg/kg. The ulcer score was the parameter measured in this model. The results revealed significant decrease in the average number of ulcers at 100 and 300 mg/kg doses for Ppa (80% and 90% respectively ANOVA $p < 0.001$). Whereas Pp at 10 and 30 mg/kg doses (20% e 80% respectively ANOVA $p < 0.05$) inhibited ulceration index. These results showed that the anti-inflammatory activity of *P. pubescens* extract has no relationship with COX inhibition or may have some selectivity for COX II. However further studies are needed to assess the antiulcerogenic mechanism of action of theses extracts.

PB18 Inhibition of detrusor contractility by a flavonoid fraction of *Bryophyllum pinnatum* – a new option to treat the overactive bladder syndrome

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Patients with overactive bladder syndrome (OAB) suffer from urgency with/without incontinence, frequency, and nocturia. Antimuscarinic agents are used as a first-line therapy with clinical benefit, but anticholinergic side effects. Patients fail to respond adequately. Therefore, we recently investigated the inhibitory effect of *Bryophyllum pinnatum* leaf press juice on porcine detrusor contractility *in vitro*. To identify the active compounds in *B. pinnatum*, we tested the effects of a flavonoid and bufadienolide fraction on the contraction of the porcine bladder strips. In a previous profiling 9 different flavonoids have been isolated and identified from the MeOH extract of *B. pinnatum* leaves. For our experiments the MeOH extract of *B. pinnatum* was partitioned between CH_2Cl_2/H_2O to separate flavonoids from lipophilic bufadienolides. The H_2O-phase was separated on a Diaion HP-20 CC to receive a flavonoid fraction. Detrusor muscle strips used for the contractility experiments were prepared from porcine bladders. In an organ bath chamber, we investigated the effect of the purified flavonoid fraction as well as of oxybutynin on the contraction of the bladder strips. The contraction was induced by Electric Field Stimulation (EFS). Flavonoid fraction concentrations of 0.7, 0.8, and 1 mg/mL reduced the contraction of bladder strips stimulated by EFS to 92.3 ± 14.3%, 60.0 ± 10.0%, and 37.0 ± 8.8%

after 74 min, respectively, of the contraction measured before treatment (100%). Concentrations < 0.7 mg/mL had no inhibitory effect, and concentrations > 1 mg/mL showed an irreversible alteration of the muscle contractility. Oxybutynin (10^{-7} M and 10^{-6} M) reduced the contraction to 27.9 ± 3.6% and 14.1 ± 3.3%, respectively. The flavonoid fraction of *B. pinnatum* inhibited the porcine detrusor contraction in a dose- and time-dependent manner. Further investigations are ongoing to study the possible synergistic inhibitory effect of the isolated bufadienolide fraction on the detrusor contractility.

PB19

Hypnosedative activity of *P. alata* and its correlation with apigenin

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Passiflora alata Curtis is a *Passiflora* species occurring predominantly in South America popularly known as sweet passion fruit. Some species of this genus are used in folk medicine due to their tranquilizing properties. Previous studies showed the importance of flavonoid compounds to the CNS activities of several species from this genus. Hence, the aim of this work was to evaluate the hypnosedative activity of the aqueous extract from pericarp of *P. alata* and its major flavonoid compound apigenin in the ethyl ether-induced hypnosis test. The extract was prepared with fresh pericarp by infusion in water (1:3 w/v). Afterwards it was filtered and freeze-dried. The quantitative analysis of the flavonoids compounds was obtained by the external standard method on a HPLC (C_{18} column; acetonitrile: 0.5% formic acid-gradient; UV detection – 340 nm, and UV spectra – 200 – 400 nm range). Groups of male Swiss mice (35 – 50 g/3 months) were p.o. treated with the extract of *P. alata* (100, 300 and 600 mg/Kg), apigenin (0.1, 0.3, 0.6 and 1.0 mfg/Kg) or vehicle (water – control group) and, 1 h later, animals were individually placed in an ethyl ether (6 mL during 13 min) saturated glass cage (20 x 15 cm). The duration of sleep (in s) was recorded. Diazepam (DZP – 1 mg/kg, p.o.) was administered to mice of the positive control group. Data were presented as mean ± S.E.M of the time (s) analyzed by one-way ANOVA followed by Dunnett's test. Apigenin was found as the major flavonoid compound in the pericarp extract of *P. alata* (0.227 ± 0.008 mg/g of extract). Considering the treatments, the aqueous extract from the pericarp of *P. alata* (600 mg/kg) and apigenin (0.6 mg/Kg) significantly enhanced the duration of sleep (Fig. 1), suggesting a hypnosedative activity. This study showed that the aqueous extract from the pericarp of *P. alata* can be effective as hypnosedative and this activity seems to be, at least in part, correlated to apigenin levels.

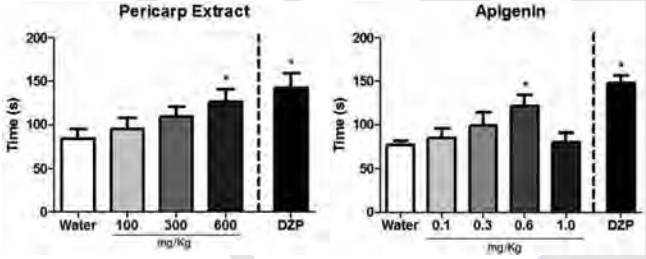

Fig. 1

Research support: FAPESC, CAPES, CNPq

PB20

Antidepressant and anxiolytic effects of methanolic root extract of *Cnestis ferruginea* Vahl ex DC (Connaraceae): Role of monoaminergic and GABAergic systems

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The root decoction of *Cnestis ferruginea* (CF) Vahl DC (Connaraceae) is used in African medicine in the treatment of psychiatric disorders [1]. This study presents the antidepressant and anxiolytic effects of methanolic root extract of CF. The antidepressant effect was studied using the forced swimming (FST) and tail suspension tests (TST) while the hole-board, elevated plus maze (EPM) and light/dark tests were used to evaluate the anxiolytic effect. Acute treatment with CF extract reduced ($P < 0.001$) the duration of immobility in FST and TST with peak effects observed at 100 mg/kg in comparison to control animals. The efficacy of the extract was found to be comparable to that of imipramine and fluoxetine (20 mg/kg; *p.o.*) in FST and TST respectively. The pretreatment of mice with metergoline (4 mg/kg, *i.p.*, a 5-HT_2 receptor antagonist) and reserpine (2 mg/kg, *i.p.*, biogenic amine depleter) 15 mins before the administration of CF (100 mg/kg; *p.o.*) prevented ($P < 0.05$) its antidepressant effect in the FST. However, pretreatment with prazosin (62.5 µg/kg, *i.p.*, α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, *i.p.*, α_2-adrenoceptor antagonist), sulpiride (50 mg/kg, *i.p.*, D_2 receptor antagonist), atropine (1 mg/kg, *i.p.*) did not prevent this effect.

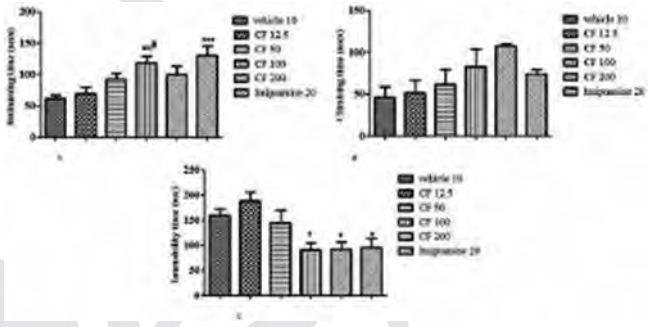

Fig. 1 A-C: Values are expressed as mean = SEM (n = 8). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control treated; $^{2}P < 0.001$ versus *C. ferruginea* 12.5 mg/kg treated.

CF (25, 50 and 100 mg/kg; *p.o*) reduced ($P < 0.05$) anxiety by increasing the number of head-dips in hole-board test, the time spent on the open arms in the EPM, and the exploration of light chamber in the light/dark test. Pretreatment with flumazenil (3 mg/kg, *i.p.*, GABA receptor antagonist) or cyproheptadine (4 mg/kg, *i.p.*) 15 min before CF (100 mg/kg; *p.o.*) reversed ($p < 0.05$) the anxiety-like behavior of the extract in EPM. It is concluded from the results obtained that *Cnestis ferruginea* produces its antidepressant effect through interaction with 5-HT_2 receptor and probably through the release of biogenic monoamines while the anxiolytic effect is produced via GABA receptor. **Reference:** [1] Garon, D., et al., (2007). Toxicon 50: 189 – 195.

PB21

Onion extract (*Allium cepa* L.) up-regulates paraoxonase 1 activity with concomitant protection against LDL oxidation in male wistar strain rats subjected to mercuric chloride induced oxidative stress

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Onion (*Allium cepa* L.) is one of the rich sources of flavonoids, consisting mainly of the major flavonols quercetin-3,4'-O-diglucoside (QDG) and quercetin-4'-O-monoglucoside (QMG) with high therapeutic properties. Paraoxonase1 (PON1) protects the oxidative modification of low density lipoprotein (LDL) and is a major anti-atherosclerotic protein component of high-density lipoprotein (HDL). We explored the roles of onion extracts and flavonoids (quercetin & catechin) in the regulation of PON1 expression by measuring its arylesterase activity and correlating with plasma MDA and oxidized LDL levels, as a marker of oxidative stress in wistar strain rats subjected to mercuric chloride ($HgCl_2$) induced oxidative insult. Rats were divided into eight groups. Control, Experimental ($HgCl_2$ treated), Exp + onion extract/catechin/quercetin, Positive control (Normal + onion/catechin/quercetin). Treatment continued for 4 weeks. PON1 activity decreased in rats treated with $HgCl_2$ ($HgCl_2$ treated 23.83 U/ml plasma; control value: 33.20 U/ml plasma) with an increase in susceptibility of LDL for oxidation (values derived after 3000 s; Pearson's r = 0.9970, $p < 0.001$; control value: Pearson's r = 0.9980, $p < 0.001$) and plasma MDA level (0.408 nmol/ml plasma; control value: 0.202 nmol/ml plasma). Onion extracts successfully and significantly attenuated the adverse effects of $HgCl_2$ by up regulating PON1 activity (28.10 U/ml plasma) and its protective capacity against LDL oxidation (Pearson's

r = 0.9978, $p < 0.001$) and lipid peroxidation (0.313 nmol/ml plasma). Similar effects were shown by quercetin and catechin with improved oxidative status either by enhancing PON1activities (31.36 & 24.76 U/ml plasma) and antioxidant defenses (0.24 & 0.28 nmol/ml plasma MDA level) or reducing susceptibility to LDL oxidation (Pearson's r = 0.9973 & 0.9984, $p < 0.001$), when given to oxidatively stressed rats.

PB22 Herbal medicinal products and gastrointestinal motility: Mechanisms of action of STW 5

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Introduction: Herbal medicinal products are in widespread use in motility-based gastrointestinal diseases, as e.g. the herbal combination product STW 5 with the indications functional dyspepsia (FD) and irritable bowel syndrome (IBS). **Aim:** After a review of the clinical data has been published recently [1], a review also of the motility-related mechanisms of action is needed. **Methodik:** A systematic database search was conducted and checked for completeness by means of hand searching and cross referencing. **Results:** There is an increasing number of publications on the herbal components of the product. The first mechanistic studies on the combination [2, 3] showed a dual mechanism of action, with a spasmolytic effect in ACh induced contractions and a tonicising effect in relaxed state. This has been confirmed [4, 5], e.g. in human intestinal sections [6] and in inflamed intestinal sections *in vitro* and *in vivo* [7 – 9]. In the stomach, a region specific action was described *in vitro* [10], which has been confirmed in a human study *in vivo* [11]. In the lower esophageal sphincter, a tonicising action has been shown *in vitro* [6], which has not yet been confirmed in a human study. **Conclusions:** The *in vitro*-, *in vivo*- and human studies showed spasmolytic as well as tonicing-prokinetic effects possibly relevant for the clinical effect of STW 5, while there are still questions open e.g. regarding the details of the cooperative effects of the components of the preparation. **References:** [1] Ottillinger et al. 2013, WMW 163:65 [2] Okpanyi et al. 1993, Acta Hort. 332:227 [3] Ammon et al. 2006, Phytomed 13 SV:67 [4] Heinle et al. 2006, Phytomed 13 SV:75 [5] Kelm et al. 2013, ZPT 34 S1:S31 [6] Schemann et al. 2008, Z Gastroenterol 46:1039 [7] Michael et al. 2009, Phytomed 16:161 [8] Sibaev et al. 2013, ZPT 34 S1:S31 [9] Wadie et al. 2012, Int J Colorectal Dis 27:1445 [10] Hohenester et al. 2004, Neurog Motil 2004, 16:765 [11] Pilichiewicz et al. 2007, Am J. Gastroenterol 102:1

PB23 Effects of *Panax ginseng* extract on osteoporosis in aged rats

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Recent research has confirmed that *Panax ginseng* (PG) has effect on cultured osteoblast of the mouse. However, the in? uence of PG on osteoporosis in animals is relatively unknown. In this study we aim to validate the usefulness of tibia quantification by correlating micro-computed tomographic (CT) images with histology analysis in the aged male rats. A total of thirty – old male WISTAR rats were used and divided into ten 8 weeks rats and ten 112 weeks aged rats with vehicle and ten 112 weeks aged rats with PG (300 mg/kg/day). Daily oral administration of PG lasted for 8weeks. Bone histomorphomeric parameters and the trabecular bone microarchitectural properties of tibia was determined by microCT scan. MicroCT analysis showed significantly lower bone mineral density (BMD) and trabecular bone number in the aged group. Ginseng prevented total BMD decrease in the tibia induced by natural aging, which was accompanied by a significant decrease in skeletal remodeling. Furthermore, the aged group with ginseng was found to have a significantly higher osteoblast. Osteocalcin concentrations were significantly lower in the 112 weeks old rats group and higher in the 112 weeks rats with PG group. Thus, further studies of the use of compounds from PG and more details of their mechanism of action for osteoporosis. The present study indicated that PG might be a potential alternative medicine for the prevention and treatment of natural aging-induced osteoporosis in human.

PB24 The extract of *Cynomorium songaricum* improves the memory disorder in rats potentially through the regulation of CREB pathway

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Alzheimer's disease (AD) is a kind of degenerative disease which is characterized by progressive intelligence deterioration. It has been demonstrated that the pCREB plays an important role to modulate the transcription of various target genes such as NGF, which participates in the processes of synapse plasticity and long term memory. Stems of *Cynomorium songaricum* have been used to treat neuronal disease traditionally in China for periods. In this study, we investigated the protective effects of the extract of *C. songaricum* against $A\beta_{1-42}$ induced injury in vivo by determination of the learning and memory ability as well as expressions of pCREB and NGF. In this study, 40 SD rats were randomly divided into 4 groups: control, model and treatment groups with donepezil hydrochloride and extract with *C. songaricum* by intragastric administration. The AD model was established by injection of agglomerated $A\beta_{1-42}$ in rat 's hippocampus. After treating 28 days, the Y-maze was used to test the learning and memory ability, immunohistochemistry and western blotting were used to test the expression changes of pCREB and NGF. The results of Y-maze showed that the total reaction time and error number of the treatment group with C. Songaricum (23.5 ± 3.35 min,10.1 ± 2.46 respectively) were significantly lower than model group (31.44 ± 5.93,16.11 ± 3.74). Immunobistochemistry and western blotting results showed the the extract of *C. songaricum* increased the expressions of pCREB and NGF comparing with the model group. Thus the extract of the C. songaricum might improve the learning and memory ability in AD rats induced by $A\beta_{1-42}$ partly due to regulate the expression of pCREB and its downstream target NGF.

PB25 Anti-allergic effect of a Korean folk medicine, KOTMIN5, *in vitro* and *in vivo*

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A Korean folk medicine, Biyeom Tang (KOTMIN5), composed of Xanthii fructus, Trichosanthis semen, Angelicae dahuricae radix, and Menthae herba, had been used in traditional medicinal clinic for the treatment of inflammatory diseases such as allergic rhinitis. However, the anti-allergic properties of KOTMIN5 and its molecular mechanisms have not been investigated. In the present study, the ethanol extract of KOTMIN5 was investigated for anti-allergic properties in bone-marrow derived mast cells (BMMC) and *in vivo* model. KOTMIN5 strongly inhibited a degranulation reaction in a dose dependent manner with an IC50 value of 17.6 mg/ml. In addition, the generation of prostaglandin D2 (PGD2) and leukotriene C4 (LTC4) was inhibited in BMMC in a concentration-dependent manner with IC50 values of 33.8 mg/ml and 24.3 mg/ml, respectively. Furthermore, KOTMIN5 reduced compound48/80-induced systemic anaphylactic shock and IgE-mediated passive cutaneous anaphylaxis in mice. These results suggested that KOTMIN5 might be useful in regulating allergic reactions.

PB26 Effect of rice bran ethanol extract on metabolic disorder in rats fed a high-fat diet

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Both water extract of rice bran and rice bran oil were previously shown to reduce fat deposition and area under the glucose-clearance curve (AUC-G) in Sprague-Dawley rats fed with high-fat diet (65% of total calories) for 4 weeks [1,2]. In order to verify the active ingredients, the 70% ethanol extract of rice bran (RBE) was studied. Rats in group 2 to 6 were high-fat fed. Group 3 to 6 were separately either co-treated daily with 3 doses (220.5, 2205, 4410 mg/kg) of RBE or 19.1 mg/kg of metformin. After 4 weeks of treatment, rats in group 2 and 3 which were co-

treated with 220.5 and 2,205 mg/kg RBE, significantly reduced the mean ± SEM of epididymal fat cell size (4310 ± 278, 4143 ± 237, *vs* 5444 ± 550 μm^2, respectively) when compared to rats those fed high-fat diet alone. However, there was no significant reduction in AUC-G, fat mass and triglyceride content of the liver. The study indicated that substances in 70% ethanol extract from rice bran had lesser potency to reduce fat mass and AUC-G than the water soluble ones. **References:** [1] Kandee N., et al (2009) Thamm Med J 9:140 – 7. [2] Charkhonpunya C., et al (2011) Thamm Med J 11:221 – 30.

PB27 Comparative antiulcer effect of curcumin and tetrahydrocurcumin in a gastric ulcer model system

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It has been reported that curcumin, a yellow pigment found mainly in rhizomes of *Curcuma longa*, accelerates ulcer healing in a chronic gastric ulcer model in rats by a main mechanism involving its anti-inflammatory activity against iNOS and TNF-a production. The suppression of iNOS production in activated macrophages and endothelium, decrease the leukocyte infiltration, a COX-2 dependent production of inflammatory PGs, and a formation of cytotoxic oxidants. Despite the promising pharmacological effects of curcumin, its poor oral systemic bioavailability limits its clinical use. The absorbed curcumin is rapidly biotransformed to its major active metabolite: tetrahydrocurcumin (THC) which is reported to be an active principle of curcumin for its antioxidant effect. In contrast, THC showed less inhibitory effects than curcumin on the induction of iNOS and COX-2 in activated RAW 264.7 cells. However, the potential gastric ulcer healing activity of THC has not been systematically examined. To investigate the possibility that the antiulcer activity of curcumin may be mediated, in part, by its active metabolites, curcumin and THC were compared in terms of their ability to accelerate the ulcer healing and to inhibit iNOS and COX-2 mRNA expression in ulcerated gastric mucosa induced by acetic acid in rats. The obtained result indicated that curcumin itself directly inhibited the production of these pro-inflammatory cytokines in the ulcerated area. In contrast, its major metabolite (THC) had less ulcer healing capacity and had no significant inhibitory effect on the activity and expression level of iNOS and COX-2. Therefore, curcumin directly exerted its gastric ulcer healing capacity and a low blood concentration of curcumin is needed to obtain an *in vivo* antiulcer effect.

PB28 Protective effect of a *Panax ginseng* extract in a rat model of cachexia

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This study investigated for the first time the possible protective effect of a standardized extract of Panax ginseng on multiple cisplatin-induced "sickness behaviors", including loss of body weight, hypothermia, malaise, hyperalgesia, fatigue, and exhaustion, in rats. These "sickness behaviors" model cancer-induced cachexia, i.e. the severely debilitating syndrome that affects the vast majority of cancer patients receiving chemotherapy. Cisplatin was administered twice weekly (1 – 2 mg/kg, i.p.) for 5 consecutive weeks (cumulative dose: 15 mg/kg). Panax ginseng extract (Ginselect®) was prepared from *Panax ginseng* C.A. Meyer Root (DER 1:3 – 5) and standardized to contain = 7.0% of ginsenosides and malonylginsenosides (= 0.9%. Rg1 < 1.4%;= 1.7% Rb1 < 3%). It was administered daily over the period of cisplatin exposure at doses of 0, 25, and 50 mg/kg (i.g.). The following parameters were recorded at baseline and at 5 weekly intervals: (a) malaise (assessed by a "yes-or-no" 6-point score for tail and paw paleness, piloerection, gastrointestinal disorders, muscle flaccidity, hindlimb weakness, and tremors); (b) body weight; (c) body temperature; (d) pain sensitivity (assessed by the Von Frey monofilament test); (e) endurance running (in a motor-driven treadmill). Treatment with cisplatin resulted in severe signs of malaise (up to a mean of 4.7 score), marked loss of body weight (up to ~15%), hypothermia (? T: ~2.3 °C), hyperalgesia (~35% reduction in nociception threshold), and ~45% reduction in duration of running time. Repeated treatment with both doses of Panax ginseng extract completely prevented all cisplatin-induced alterations at all recording times. These data indicate a remarkable protective effect in a rat model of cachexia and suggest that the extract may have a great potential as supportive care in oncology. A clinical trial with Ginselect® is under completation at MD Anderson Cancer Center (Houston, TX, USA) for cancer-related fatigue in 158 oncological patients.

PB29 Accelerated remission of symptoms of acute viral rhinosinusitis with a dry extract of five herbal drugs (BNO 1016)

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Background: BNO 1016 is a dry extract composed of the five herbal drugs Gentian root, Primula flower, Sorrel herb, Elder flower and Verbena herb (ratio 3:3:3:3:1). Clinical efficacy of BNO 1016 was proven in a clinical trial (ARhiSi-2) by visualising a significant group difference regarding the major symptom score (MSS) at the end of treatment[1]. Here we show the group difference for the per protocol (PP) analysis set and how the difference translates into an acceleration of disease remission. **Methods:** In this randomised placebo-controlled clinical trial in line with EPOS 2012 386 patients with sonographically confirmed acute viral rhinosinusitis were treated with 480 mg BNO 1016 or placebo for a period of 15 days. Primary efficacy criterion was the investigator assessed mean MSS at end of treatment. A difference of 1 score point was prospectively defined as clinically relevant. Additionally, it was analysed how much faster remission of symptoms occurred in the BNO 1016-treated group compared to placebo. **Results:** 300 patients were analysed in the PP analysis set (BNO 1016: n = 147; placebo: n = 153). The MSS at end of treatment was 3.47 ± 0.28 for placebo and 2.07 ± 0.18 for the BNO 1016-treated group, resulting in a group difference of 1.40 ± 0.28 score points at end of treatment ($p < 0.0001$). In the BNO 1016-treated group a MSS of 3.47 was reached approximately 3.8 days earlier than in the placebo treated group. **Conclusion:** BNO 1016 demonstrated a significant and clinically relevant improvement of symptoms, documented by a group difference of 1.40 score points at end of treatment. Remission of symptoms for the BNO 1016-treated group is accelerated by 3.8 days compared to placebo. **Reference:** [1] Jund R et al. *Rhinology online 2012*

PB30 *In vivo* effects of green and black tea on the rat male reproductive system

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Green (unfermented, GT) and black (fermented, BT) tea (*Camellia sinensis*) may improve male reproductive functions due to their high level of antioxidants. In this study, Male rats received GT or BT (2% or 5%, 5 min brewed) as sole source of drinking for 52 d in order to investigate the effect on the male reproductive system. Chemical analysis showed that GT had higher levels of soluble solids (SS), total polyphenol (33.3 vs. 25.9% of SS for BT) and flavanol (29.5 vs. 6.1% of SS for BT) while BT contains more flavonol. Both teas had no significant effect on serum antioxidant capacity, body weight gain, relative weight of reproductive organs and liver. However, the 5% teas increased relative kidney weight and creatinine activity. In contrast, activities of liver markers, ALT and AST, were significantly reduced. Testis showed mainly a normal spermatogenesis, although a significant decrease in the tubule diameter and germinal epithelial height was noticed. Also, GT and BT significantly increased epithelial height of the epididymis. Epididymal sperm concentration was significantly increased after GT treatment. Both teas significantly enhanced sperm viability and total motility. In addition, 2% black tea also significantly raised progressive sperm motility. GT and BT trea-

ted groups displayed a significant increase in spontaneous acrosome reaction from 5.25% to 12% and 14%, respectively. Serum testosterone level showed a significant concentration-dependent increase from 5.3 to 8.73 ng/ml after exposure to GT. In contrast, black tea lowered testosterone levels to 3.2 ng/ml. In conclusion, green and black tea significantly improved sperm concentration, viability and motility. This is beneficial for male fertility and might be due to the high level of antioxidants. However, the increased level of acrosome reaction might be a reason for concern as this would inhibit the fertilization process. Intake of large amounts of GT or BT may also impair kidney function.

PB31

Intake of *Crataegus* extract WS® 1442 compared to physical exercise in mildly overweight, healthy volunteers – safety and influence on endothelial function

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Aims: Safety and efficacy of Crataegus extract WS® 1442 have already been demonstrated in patients with NYHA II/III cardiac insufficiency. A pilot study was performed to investigate the safety of two different doses of WS® 1442 in mildly overweight subjects, including preliminary investigations on the influence of the drug on endothelial function and lipid parameters. **Methods:** 60 male or female mildly overweight (BMI: 25.0 – 29.9 kg/m^2) otherwise healthy volunteers (age range 45 – 75 years) were randomised to receive WS® 1442 450 mg b.i.d., WS® 1442 900 mg b.i.d., light exercise (Nordic Walking, 2 x 30 min/week) or moderate exercise (Nordic Walking, 4 x 45 min/week) for 12 weeks. As exploratory pharmacodynamic outcome, parameters of endothelial function (Reactive Hyperaemia Index [RHI]; Augmentation Index [AI]) and plasma lipids were measured. Furthermore, quantitative measurement of endothelial progenitor cells (EPC) was conducted. **Results:** In all groups, subjects with impaired endothelial function at baseline (RHI< 1.67) showed improvements of RHI. Overall, the higher dose of WS® 1442 (900 mg b.i.d.) did not show additional benefit compared to the currently recommended daily dose. An increase in maximal duration of exercise, workload and exercise capacity was only seen in the Nordic walking groups. All reported adverse events were either not or improbably related to the investigational treatments. **Conclusions:** WS® 1442 was safe and showed promising effects in slightly overweight, healthy volunteers.

PB32

Pharmacokinetics of linalool and linalyl acetate in rats after repeated oral administration of silexan, an essential oil from *Lavandula angustifolia* flowers

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Silexan is the active ingredient in Lasea® – an approved drug for the treatment of restlessness and mild anxiety in Germany. The naturally occurring enantiomers R-(-)linalool (L) and R-(-)-linalyl acetate (LA) are the main constituents of silexan representing 70 – 80% of the total oil. In previous studies we have reported on the pharmacokinetics of L and LA in rats after single oral administration of silexan or the two major individual constituents (Nöldner et al., 2011). We now investigated the pharmacokinetics of L and LA after repeated application of silexan (100 mg/kg/day p.o.) over a period of 14 consecutive days. The plasma and organ concentrations of L and LA were measured by headspace GC-MS for 48 h after the last administration.

	Linalool				Linalyl acetate			
	Single dose		Repeated doses		Single dose		Repeated doses	
Tissue	Cmax (ng/ml or g)	Tmax (h)	Cmax (ng/ml or g)	Tmax (h)	Cmax (ng/ml or g)	Tmax (h)	Cmax (ng/ml or g)	Tmax (h)
Plasma	77	0.25	108	0.25	n.d.	-	n.d.	-
Brain	164	0.25	140	0.25	31	0.25	48	0.25
Liver	2287	0.25	2095	0.25	n.d.	-	25	32
Kidney	670	0.25	1547	0.25	n.d.	-	12	4
Fat	2085	4	3958	4	n.d.	-	117	2

n.d.: not detectable

The results show that both single and multiple doses of silexan result in similar concentrations of L in plasma and all analyzed organs. However, the concentrations and distribution of LA were significantly different. After single administration LA was only measured in the brain while it was detected in all tissues except the plasma after repeated administration although at low concentrations. The lower LA levels are obviously due to a very quick metabolization of LA into L. In conclusion, the results demonstrate that oral administration of silexan rapidly results in high plasma and tissue levels of L without a clear tendency for accumulation after repeated application. **References:** [1] M. Nöldner, S. Germer, E. Koch (2011). Planta Med. 77, 1410.

PB33

The phytochemical and anti-nociceptive evaluation of the methanol extract of *Newbouldia laevis* (Bignoniaceae) leaves using animal models

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This present study was carried out to evaluate the anti-nociceptive activity of *Newbouldia laevis* leaves extract (NLLE) using acetic acid induced writhings in mice, hot plate and tail-flick test. The leaf, stem, stem bark and fruits have been used for febrifuge, stomach ache and tooth ache (Iwu, 2000; Lewis and Manony, 1997). The phytochemical screening and acute toxicity was studied. A significant ($P < 0.050$ increase in the latency period in the tail flick and hot plate test was observed for the treatment with NLLE at dose of 100, 200 and 400 mg/kg b.wt. i.p after 30 and 45 min. In hot plate method, the percentage observed after 45 min was 48.31, 67.05, 86.16 and 89.17 for NNLE at the 100, 200 and 4000 mg/kg b.wt. and piroxicam (20 mg/kg) respectively while in tail flick method, 28.04, 41.66 and 60.85% reaction time was observed with 100, 200 and 400 mg/kg b.wt. of NLLE. A 31.92, 53.58 AND 67.47% ($P < 0.05$) inhibition of writhing was observed with NLLE at 100, 200 and 400 mg/kg b.wt.i.p which was found comparable to piroxicam (20 mg/kg) which inhibited 72.38% ($P < 0.05$) of writhing reflex. Acute toxicity studies showed it has a wide margin of safety and good tolerance at 5000 mg dose. The phytochemical analysis of the extract revealed the presence of carbohydrates, alkaloids, resins, steroids and flavonoids. The result showed that the methanol extract of *Newbouldia laevis* possesses anti-nociceptive activity which may be mediated by the central and peripheral mechanisms and supports the traditional uses of the plant in the treatment of pains and inflammation. **References:** [1] Iwu, MM (2000). Handbook of African Medicinal Plants. London: CRC, Press, Inc; p. 19. [2] Lewis, WH, Manony, PFE (1997). Medical Botany: Plants Affecting Man's Health. New York, USA. Johny Wiley and Sons, p. 240.

PB34

Potential of Peruviose A and B from *Physalis peruviana* L calyces to treat Inflammatory Bowel Disease: *In vivo* and *in vitro* studies

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Phytotherapy constitutes an emerging alternative strategy for the treatment of inflammatory bowel disease (IBD). Although medicinal plants are integral part of Colombian culture, very few of them have been studied deeply.[1] One example is *Physalis peruviana* (Cape gooseberry), which has demonstrated anti-inflammatory activity.[2] In this work, we investigated the therapeutic potential of Peruviose A and B; two new sucrose esters recently isolated by us from calyces of *P. peruviana*, using TNBS-colitis model. For this, ten rats were assigned to groups: control, untreated TNBS-colitis, and treated with a mixture of Peruviose A and B (5 and 10 mg/kg, i.p.) for two weeks. Inflammation was evaluated measuring macroscopic/histologic damage, MPO activity, and cytokine levels. *In vitro* studies were performed using LPS-stimulated peritoneal macrophages treated with compounds (0.01 – 10 µg/mL). Complementarily, we assessed their effect on *E. coli* growth. Treatment with Peruviose A/B, at both doses, did not show hepatic or renal injury, while reduced significantly the extent and severity of tissue damage, and colonic

weight/length ratio by 58%. In agreement, microscopic disturbances were diminished with reduction of inflammatory cells, distortion of crypts architecture, and necrosis. Compounds also reduced MPO activity (71%), TNF-α (33%) and IL-1β (64%) levels, while IFN-γ and IL-6 were not modified. *In vitro* assays showed a dose-response inhibition of NO and PGE2 liberation (IC_{50}= 3.9 and 0.07 µg/mL, respectively). Additionally, compounds did not affect *E. coli* growth, suggesting that the activity is not related with effects on commensal bacteria. Our results provide the first evidence that Peruviose A/B can effectively ameliorate experimental IBD, giving a new application to calyces of *P. peruviana*, which constitutes a waste in fruit production and an unexplored source of bioactive molecules. **References:** [1] Gomez *et al. J Ethnobiol Ethnomed*. 2011;7:27. [2] Franco *et al. Biomédica*. 2007; 27:110 – 115.

PB35 Wound healing and anti-ulcerogenic activity of *Gardenia angustifolia* extract in rats

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Gardenia angustifolia (*Rubiaceae)* is widely utilized in many parts of Nigeria to manage a wide range of ailments. As part of efforts to elucidate its pharmacological activities and hence medicinal potential, wound healing and anti-ulcerogenic properties of the methanol extract was evaluated using experimentally created wound and ulcers in albino rats. Wound healing properties was evaluated using excision wound model, while anti-ulcer activity was studied using ethanol induced ulcer model. Five groups of rats were experimentally wounded at the back area. An area of uniform wound of 7 x 7 mm using millimeter ruler was excised. The animal groups were topically treated with *G. angustifolia* gel, wound dressed with leaf, fruit and root gel significantly healed earlier than those treated with paraffin base and povidone iodine (standard). In anti-ulcer studies, rats were orally administered with different doses of the root extract (100, 250 and 500 mg/kg body weight) and positive control group (Omeprazole, 8 mg/kg body weight) for five days. After induction of ulcer using 5 ml/kg body weight of ethanol, the stomachs of the rats were opened; gastric volume and ulcer area were measured. The results indicate that *G. angustifolia* root extract can prevent ulceration in rats in a dose dependent manner. The acute toxicity study revealed that the plant was toxic at higher doses (5000 mg/kg body weight). Blood glucose reduction was dose and time dependent. Based on this study it becomes evident that *G. angustifolia* exhibits in rats anti-ulcer and also significant wound healing accelerating properties, of wounds dressed with the root, leaf and fruit gels.

PB36 Evaluation of hepatoprotective activity of the *Acacia nilotica* flowers on carbon tetrachloride-induced liver damage in rats

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The hepatoprotective effect of aqueous methanol extract (MeOH: H_2O, 7:3) of *Acacia nilotic* flowers was investigated against carbon tetrachloride-induced hepatotoxicity in rat. The extract was tested in two dose levels 20 and 40 mg/kg b.wt., for 4 weeks versus silymarin (25 mg/kg), samples were taken after 2, 3 and 4 weeks of treatment. Serum aspartate (AST), alanine aminotransferases (ALT) and alkaline phosphatase (ALP) CCl_4-treatment group were elevated to be: 97.4 ± 2.11; 57.8 ± 1.65 U/L and 71.3 ± 2.3 IU/dl; respectively comparing with the control values. Treatment with the plant extract (20 and 40 mg/kg) significantly reduced ALT, AST and ALP in dose dependant manner after 4 weeks in the CCl_4-intoxicated rats. Also, *A. nilotic* significantly increased the activities of SOD (19.4 ± 0.98; 22.1 ± 1.25; 21.7 ± 1.54 U/mg protein), CAT (3.24 ± 0.043; 3.85 ± 0.06; 4.32 ± 0.05 U/min), GPx (65.3 ± 2.11, 73.5 ± 2.18, 77.0 ± 2.24 nmol/mg protein), GSH (412 ±.7.2; 543 ± 5.41; 731 ± 8.6 mol./mg tissue), while MDA decreased significantly (71.2 ± 1.33; 78.5 ± 1.72; 79.4 ± 1.92 nmol/mg protein) in lower and higher dose and silymarin, respectively compared with CCl_4-treatment. Microscopic examination of CCl_4 treated animals revealed focal necrosis and lymphocytic infiltration in the periportal areas with massive fatty infiltration. The histopathological examination showed that extract markedly reduced the alterations induced by CCl_4 in liver. In addition, *A. nilotica* decreased inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) activation in CCl_4-treated rats. Phenolic compounds are commonly found in plants and have been shown to display remarkably of biological activities, such as hepatoprotective, anti-inflammatory and antiviral activities. Therefore, our findings suggest that the phenolic compounds are the major active compounds responsible for the hepatoprotective activity of *A. nilotica* flowers.

PB37 Neuroprotective efficacy of *Punica granatum* peels extract against rotenone induced oxidative stress in rat brain

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Punica granatum has the highest free radical scavenging capacity among the tested medicinal plants which are being used traditionally for the treatment of large variety of several diseases (1). The present study was aimed to investigate the antioxidant and neuroprotective effects of *P. granatum* peels aqueous methanol extract against rotenone-neurotoxicity in rat. The animals pretreated with *P. granatum* peels extract 75 and 150 mg/kg 60 min before of rotenone injection (2 mg/kg) for 4 weeks. Rotenone significantly reduced dopamine, glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) contents and locomotors activity; as well as, It increased malondialdehyde (MDA) level in the brain homogenate. *P. granatum* significantly reversed the reduction of antioxidant enzyme system toward the normal values in dose dependant manner. Histopathological examination indicated severe damage in cortex and striatum; this damage significantly attenuated by *P. granatum* peels extract. In addition, immunohistochemical studies demonstrated that *P. granatum* peels extract-treated groups had significant suppression in caspase-3, cyclooxygenase-2 and inducible nitric oxide synthase. Conclusion: *P. granatum* peels extract could ameliorate brain oxidative stress induced by rotenone via upregulating the antioxidant defense mechanism and by attenuating lipid peroxidation. *P. granatum* peels extract thus may be used as potential therapeutic agent in preventing neurodegenerative diseases.

Tab. 1: Levels of lipid peroxides (LPO). reduced glutathione (OSH) and the activities of glutathione peroxidase (GPx), glutathione-Stransferase (GST), catalase (CAT) and superoxide dismutase (SOD) in the cerebellar tissue of normal and experimental groups of rats treated with Rotenone (2 mg/kg) and *P. granatum* (75 & 150 mg/kg) for 4 weeks.

Parameters	Control	Rotenone	*P. granatum* (75 mg/kg) + Rotenone	*P. granatum* (150 mg/kg) + Rotenone
Dopamine ng/mg tissue	2.31 ± 0.03^{A}	1.42 ± 0.07^{B}	2.24 ± 0.05^{C}	2.63 ± 0.08^{A}
GSH ug/g tissue GPX U/g tissue	2.62 ± 0.06^{A} 275.3 ± 1.90^{A}	1.31 ± 0.05^{B} 192.11 ± 2.11^{B}	2.77 ± 0.04^{AC} 250.15 ± 2.50^{C}	2.84 ± 0.07^{C} 280 20 ± 2.31^{A}
GST ug/g tissue SOD U g/g tissue	1280 ± 18.21^{A} 14.32+ 0.38^{A}	987 ± 11.3^{B} 12.45 ± 0.29^{B}	1053 ± 14.1^{C} 15.7 ± 0.34^{C}	1345 ± 17.2^{D} 18.1 ± 0.42^{C}
CAT U/g tissue MDA N g/g tissue	12.3 ± 1.22* 135.2 ± 2.78^{A}	8.10 ± 1.1^{B} 278.1 ± 3.22^{B}	13.7 ± 1.9^{C} 171.8 ± 3.51^{C}	15.6 ± 0.81^{C} 148. 2 ± 2.91^{D}
Locomotor *	180 ± 2.2^{A}	12.11 ± 1.3^{B}	150.1 ± 2.4^{C}	177 ± 2.4^{A}

ANOVA – one way (the different capital letters are significantly different at P< 0.05).
Data represent mean values (± SE) of 8 rats per group
*Locomotor activity: Number of spontaneous movements/6 minutes (second).

References: [1] Singh et. al. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *J Agric Food Chem*, **50**:81 – 6.

PB38 Effects of oral administration of ethanolic extract of *Vernonia amygdalina* on enzymes associated with liver function in experimental rabbits

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Vernonia amygdalina Delile has been used ethnomedicinally in the Southern Nigeria and in most parts of Africa in the treatment of Diabetes mellitus, Malaria and Typhoid. It is also eaten as vegetable in most local delicacies in these parts. Most of these ethnomedicinal uses have been validated, without considering toxicity aspects. Thus the effects of oral administration of ethanolic extract of *Vernonia amygdalina* Delile on enzymes associated with liver function in experimental rabbits were studied. The animals were divided into three groups viz: I, II, and III. Group I was the control, groups II and III were the study groups. Each group comprised two male and two female rabbits. Groups II and III were administered with 100 mg/kg and 500 mg/kg doses of the extract respectively. Enzyme assay in study groups II and III, alanine aminotransferase showed an increased activity from 39.75 IU/L – 50.00 IU/L for males and 37.75 IU/L- 50.00 IU/L for females as against 1.00 IU/L and 9.00 IU/L seen in the control males and females respectively. The liver damage is further seen as suggested by increased bilurubin levels ranging from 35.75 µmol/L to 48.10 µmol/L in males as against 12.95 µmol/L of control males and 49.95 µmol/L to 57.08 µmol/L in females compared to 12.03 µmol/L of control females. An increase in relative liver weights was seen in groups II and III ranging from 0.68% to 1.60% in males and 0.56% to 0.48% in females compared to 0.15% and 0.25% in male and female control rats, indicating no disease condition. Reduction in spleen weights was observed as seen in the decreased spleen mean relative weights of 4.47 x 10 – 4 Kg and 6.02 x 10 – 4 Kg in study groups 1 and 2 respectively as against 8.04 x 10 – 4 Kg in the control. *Vernonia amygdalina* Delile despite its constant use in local delicacies and ethnomedicines has shown some indication of toxicity. The benefits of its uses should be weighed against its toxicity.

PB39 Ferruginol from *Prumnupitys andina*: absolute configuration and anti-inflammatory activity

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Ferruginol, a type-abietane diterpenoid, previously obtained from *Prumnupitys andina* has displayed to possess an interesting range of biological activities such as *in vitro* anti-inflammatory activity, cardioprotective, antifungal, antiplasmodial, antimalarial, antibacterial, antioxidant, anti-tumoral and gastroprotective.[1] Aim of this research is corroborate the absolute configuration of ferruginol by quantum calculation of specific rotation values. Anti-inflammatory dermal activity of ferruginol, is reported by first time, using in vivo models. Ferruginol was subjected to topical assays for the inhibition of murine ear inflammation elicited by arachidonic acid (AA) or phorbol ester (TPA).[2] The topical anti-inflammatory activity was evaluated *in vivo* using groups of 8 animals were treated with ferruginol (equimolar doses with regard to the reference drug Indomethacin), dissolved in acetone and applied topically on the inner (10 mL) and outer (10 mL) surfaces of the right ear of the animals of each group. We report the absolute configuration of ferruginol by quantum calculation of specific rotation values and its topical anti-inflammatory activity. Ferruginol was subjected to topical assays for the inhibition of inflammation elicited by arachidonic acid (AA) or phorbol ester (TPA). Ferruginol showed topical anti-inflammatory activity in both AA and TPA models (Table 1). The anti-inflammatory effects were dose-dependent. Its maximal effect observed against TPA (20,5%) and AA (62,5%). **References:** [1] *Areche C, San-Martin A, Rovirosa J, Muñoz MA, Hernández-Barragán A, Bucio MA, Joseph-Nathan, P.* J. Nat. Prod. **2010;** 73: 79. [2] *Rodríguez-Díaz M, Delporte C, Cassels BK, González P, Silva X, León F, and Wessjohann L.* J Pharm Pharmacol. **2011**; 63: 718 – 724.

PB40 Renoprotective effect of grape seed extract against oxidative stress induced by gentamicin and hypercholesterolemia in rats

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Kidneys are dynamic organs and represent one of the major systems maintaining the body homeostasis; they are affected by many chemicals and drugs. Grape seed extract (GSE) has been targeted to prevent drug-induced renal toxicity. This study investigates the possible renoprotective effect of GSE against oxidative stress, renal impairment, and hypercholesterolemia (HC) induced by gentamicin (GM) and cholesterol-enriched diet. Seventy adult male Wistar rats (160 ± 10 g) were divided into seven groups: (1) served as control, (2) GSE, (3) GM, (4) GSE + GM, (5) hypercholesterolemic (HC) group, (6) GM + HC group, and (7) GM + HC + GSE. Kidney functions, inflammatory mediators, cytokines, lipid profile, nitric oxide (NO), cyclic guanosine monophosphate (cGMP), and oxidative and antioxidative stress parameters were assessed in all groups. GM induced renal dysfunction, which was exacerbated by the presence of HC as confirmed by laboratory determinations. Administration of GSE attenuated the renal toxicity evidenced in significant reduction in elevated kidney function, inflammatory cytokines as well as lipid profile, NO, cGMP, enzymatic, and nonenzymatic antioxidants. Conclusion: Administration of GSE simultaneously with GM attenuated oxidative stress, diminished renal toxicity, and improved lipid profile induced by GM and HC.

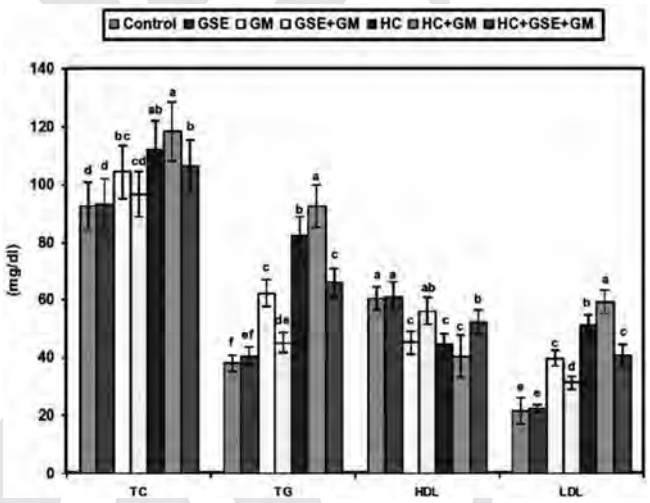

Fig. 1: Effect of GSE on lipid profile of hypercholesterolemic rats treated with gentamicin.
*Columns followed by the same alphabetical letter for each parameter are not significantly different at P < 0.05.

PB41 *Portulaca oleracea* L. exhibits increased memory ability in rats

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Alzheimer disease (AD) is a complex mental illness, which is characterized by an age-dependent loss of memory. Natural products are one of the most probable sources for discovering effective treatments for neurodegenerative disorders (ND). Many studies have shown that natural antioxidants from plant sources can effectively reduce the risk of ND. *Portulaca oleracea* L. is used in traditional Persian medicine to support the brain. The protective effect of *P. oleracea* was tested already *in vitro* and considerable results were obtained from the oxidative stress test on PC12 nerve cells (MTT cell viability assay ($p < 0.0005$)) [1]. The aim of our study was to investigate potential activity for pretreatment of ND. Aerial parts of *P. oleracea* were collected in Iran and air dried under shade for 4 weeks. Crushed drug was extracted by maceration (20 °C) using 70% aqueous ethanol for 48 hours. The dried semisolid POEE (*Portulaca oleracea* ethanolic extract, yield 11.4%) was then prepared

for gavage feeding. Wistar rats were divided into five groups and were treated daily for 14 days as follows: Group-1 was nourished with normal water (control). Four other groups received EEPO orally at the doses of 100-, 200-, 400- and 800 mg/kg, respectively. The spatial memory and the learning ability were tested by using the Morris Water Maze method (MWM). POEE showed significant dose dependent effects at the dose of 400 mg/kg (p = 0.004) and 800 mg/kg (p = 0.03) by improving the memory capability. This extract may be an option for pretreatment of AD because of the already known ability to reduce oxidative stress on nerves cells plus the finding that it shows efficacy on memory ability in rats.

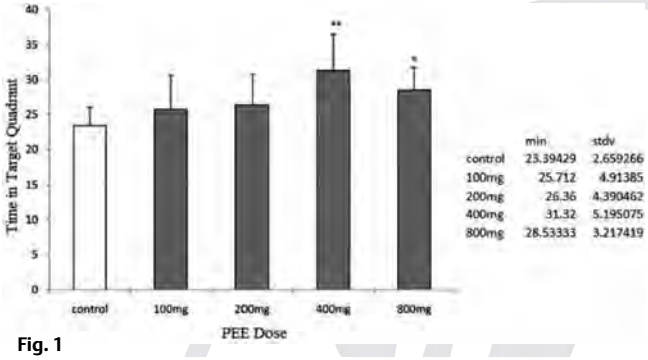

Fig. 1

Tab. 1: Multiple Comparisons

Dependent Variable: prob							
			Mean Difference			95% Confidence Interval	
	(I) group	(J) group	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
	4	1	7.92571*	2.50754	.004	2.7385	13.1130
LSD							
		2	560800*	2.68372	.048	.0563	11 1597
		3	4.96000	2.58241	.067	-.3821	10.3021
		5	2 78667	2.58241	292	-2 5555	8 1288
	5	1	513905*	2 22576	.030	.5347	9 7434
		2	2 82133	242252	256	-2 1900	7.8327
		3	2 17333	2.30978	357	-2 6048	6 9515
		4	-2 78667	2 58241	292	-8 1288	2 5555

Group 1: cont.
Group 2: 100 mg/kg
Group 3: 200 mg/kg
Group 4: 400 mg/kg
Group 5: 800 mg/kg

Reference: [1] Shabnam Sarshar and Hasan Rafati, Neuroprotective effects of portulaca total extract and its major fractions, 15th International Congress "PHYTOPHARM 2011" abstracts book, 95 – 96

PB42 *Crataegus* special extract WS® 1442 ameliorates angiotensin II-induced endothelial dysfunction in rats

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Impaired endothelium-dependent relaxation is a characteristic hallmark of most cardiovascular disorders such as hypertension, atherosclerosis, coronary/peripheral artery disease or chronic heart failure. Indeed, endothelial dysfunction (ED) has been shown to be of prognostic value in predicting vascular events including stroke and myocardial infarction. A major cause for diminished vasodilatory responses is a reduced endothelial generation of nitric oxide (NO) or its accelerated degradation due to oxidative excess. WS® 1442 is a dry extract from hawthorn (Crataegus ssp.) leaves with flowers that previously has been reported to induce endothelium-dependent NO-mediated relaxations of porcine coronary artery rings through a stimulation of endothelial nitric oxide synthase (eNOS). It was the aim of the present study to evaluate whether intake of WS® 1442 is also able to prevent ED in vivo. For this purpose, ED was induced in male Wistar rats by infusion of angiotensin II (Ang II, 0.4 mg/kg/day) for a period of 14 days using implanted osmotic minipumps. WS® 1442 (about 300 mg/kg/day) was administered via enriched feed for one week before and during the Ang II infusion. Following euthanasia reactivity of the isolated aorta was assessed by relaxation of the phenylephrine-precontracted vessel to increasing concentrations of acetylcholine. In addition, the total oxidative capacity, prorenin/renin concentration, and renin activity were determined in the plasma. Ang II infusion induced an impaired relaxation of the aorta and decreased renin activity which were almost completely or partially normalized by treatment with WS® 1442. However, WS® 1442 had no effect on the decreased oxidative capacity and the reduced prorenin/renin concentration. In conclusion, the present findings indicate that intake of WS® 1442 improves Ang II-induced ED in rats. This protective effect is most probably due to an enhanced endothelial formation of NO following activation of eNOS.

PB43 Protective role of curcumin in deltamethrin induced system toxicity in Wistar rats

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The current study was performed to assess the adverse effect of deltamethrin on testes, liver and kidney in male rats and to evaluate the protective role of curcumin in alleviating the detrimental effect of deltamethrin on these organs. Twenty four male Wistar rats (150 – 200 g) were divided in four groups (6 rats each): group I was taken as control, group II received deltamethrin (6 mg/kg), group III deltamethrin (6 mg/kg) along with curcumin (100 mg/kg) and group IV curcumin (100 mg/kg). Deltamethrin and curcumin were administered orally by gavaging for 28 days. Deltamethrin caused a significant reduction in reproductive organ weights, sperm count, sperm motility, serum testosterone (T), follicle stimulating hormones (FSH) and luteinizing hormones (LH). Glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione S transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) were decreased in testis, liver and kidney of exposed rats. Deltamethrin exposure significantly increased liver and kidney weight, sperm abnormalities and lipid peroxidation (LPO) level. Deltamethrin also caused histological alterations in testes, liver and kidney in rats. In group III, treatment with curcumin improved reproductive organs weights, sperm characteristics and hormones levels. Deltamethrin induced oxidative damage and histopathological alterations in testes, liver and kidney were also recovered. Results indicate that deltamethrin exerts significant harmful effects on testes, liver and kidney. Concurrent administration of curcumin partly reduced the detrimental effects of deltamethrin on these organs. Curcumin treatment group showed increase in sperm characteristics, reproductive hormones levels, enzymatic and non-enzymatic antioxidants and decrease in lipid peroxidation level and preserved the normal histological architecture of the testes, liver and kidney. **Keywords:** Deltamethrin, curcumin, system toxicity, Wistar rats, histology

PB44 Effects of lipid extract of sea urchins gonads in metabolic syndrome animal model

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Insulin resistance associated with visceral fat obesity has been suggested to be the pathological basis of metabolic syndrome. Many studies have demonstrated increased oxidant stress in diabetic patients and animal models of diabetes mellitus. In this study, the effect of the administration of lipid extracts of sea urchin gonads (LESUG) on blood glucose levels was examined in SHR rats that show spontaneously occurring metabolic syndrome-like abnormalities. Diabetes was induced by intraperitoneal injection of streptozotocin and nicotinamide. Rats were treated *per os* by LESUG (0.37 – 1.11 mg/kg/day) for 26 days. The levels of fasting blood glucose and HbA1c were determined 3 days before and 26 days after pathology induction. The LESUG showed significant effects on the fasting blood glucose level and tended to inhibit the increase of HbA1c (66% and 54% in a dose of 1.11 mg/kg compared with the control group, respectively). In all experimental groups, no change was seen in body weight gain compared with untreated groups throughout the experimental period, whereas in the untreated group significant increases were observed. It was found that the administration of the lipid extract of sea urchins gonads resulted in normalization of oxidative status. The levels of reduced glutathione and malonic aldehyde were not different from those of intact animals, and the normalization of these parameters correlated with a decrease in blood glucose levels. Adminis-

tration of the LESUG in doses of 0.37, 0.74, and 1.11 mg/kg resulted in a dose-dependent reduction of blood pressure in SHR rats (14, 18 and 19% respectively, compared to the control group).

PB45 Inhibitory effect of *Solanum tuberosum* L. var. Vitelotte extract in the development of 2,4-Dinitrochlorobenzene-induced atopic dermatitis in NC/Nga mice

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Solanum tuberosum L. var. Vitelotte (SV) contains various polyphenols and anthocyanins that are believed to have antioxidant and anti-inflammatory activity. The objective of this study is to evaluate the effects of oral administration of SV extract on 2,4-dinitrochlorobenzene (DNCB)-induced atopic dermatitis (AD) in NC/Nga mice. The DNCB-induced NC/Nga mice were used for treatment with SV extract (75, 150, and 300 mg/kg) for 4 weeks. Oral administration of SV extract alleviated the AD-like skin symptoms, scratching behaviors and ear thickness. Oral administration of SV extract reduced the level of IgE and IgG1, whereas it increased the level of IgG2a in a dose-dependent manner [1]. The calculated IgG1/IgG2a ratio for each mouse revealed that the SV extract also significantly reduced the Th2/Th1 ratio, IL-4 and IL-13 (as Th2 cytokines), IFN-gamma and IL-12 (as a Th1 cytokines) in spleens [1]. In addition, it significantly decreased gene expression such as eotaxin-1, CCR3, IL-4, IL-5, IFN-gamma, IL-12, and TARC, in AD-like lesions. Histological analyses demonstrated decreased thickening of the epidermis as well as dermal infiltration by inflammatory cells [2]. These results suggest that SV extract can be considered as candidate for therapeutic agents for the treatment of AD via an immune-regulative effect. **Reference:** [1] Kim, M.J. et al. (2012) Evid-based Compl. Alt. Epub 2012 Oct 23. [2] Choi, J.H. et al. (2012) Food. Chem. Toxicol. 50: 2923 – 2929.

PB46 Effects of *Momordica dioica* aqueous extract in alloxanised diabetic rats and secretion from isolated pancreatic islets

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Antidiabetic activity of extracts of *Momordica dioica* fruit was evaluated in alloxanised diabetic rats to verify its folkloric use in management of diabetes. Also, the *in vivo* and *in vitro* insulinotropic effects of the most active extract were also determined. Its aqueous extract (AEMD) elicited maximum (52.8%) blood glucose reduction at 1 hour in glucose tolerance test (GTT), which was significantly ($p < 0.01$) lower than 39.0, 37.2 and 37.7% given by hexane, chloroform and ethanol extracts, respectively. The most active AMED (200 mg/kg bw) produced a significant ($p < 0.01$) fall of 57.5% in alloxanised diabetic rats while its 200 mg/kg bw AMED once daily administration for 21 days reduced the elevated blood glucose, post prandial glucose and glycosylated hemoglobin by 64.8, 76.9 and 37.6%, respectively. It also gave a 31.3% increase in serum insulin levels, suggesting that its antidiabetic effect may be due to its pancreatic and serum insulinotropic actions. Effects of AEMD, alone or in combination with 1 mg of nicorandil, on isolated islets of normal Wistar rats incubated in HBBS buffer at 3.3 and 16.7mM glucose were assayed. AEMD (1 and 2 mg) dose dependent stimulated insulin secretion and the induced enhancement was not diminished by nicorandil, suggesting that the insulin induction was independent of K-ATP channels of β cells. **Keywords:** *Momordica dioica*, antidiabetic, Wistar rat, pancreatic islets, insulin, nicorandil

PB47 Anti-inflammatory Activity Researches on *Tripleurospermum parviflorum* (Willd.) Pobed. and *Tripleurospermum tenuifolium* (Kit.)

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Tripleurospermum species have been used for the treatment of inflammatory diseases in Turkish traditional medicine. The present study was designed to evaluate the anti-inflammatory activity potential and fatty acid composition of the extracts prepared from the aerial parts of *Tripleurospermum parviflorum* (Willd.) Pobed. and *T. tenuifolium* (Kit.) Anti-inflammatory activity was assessed by using carrageenan-, and serotonin- induced hind paw edema and acetic acid-induced increase in capillary permeability models. EtOAc extracts of *T. parviflorum* and *T. tenuifolium* exerted remarkable inhibitory effect in the carrageenan- induced hind paw edema model with the values of 27.4 and 24.4%, respectively. The EtOAc extracts of *T. parviflorum* and *T. tenuifolium* also demonstrated notable anti-inflammatory activity on acetic acid-induced increase in capillary permeability model with the significant inhibition values of 29.3 and 26.3%, respectively at 200 mg/kg dose. The fatty acid compositions of the plants were investigated by gas chromatography (GC). Generally, C 16:0 palmitic acid and C 18:2 linoleic acid were found to be the major fatty acids in two species. Saturated fatty acids (SFAs) were found in higher amounts than monosaturated and polyunsaturated fatty acids. SFAs were determined as 58.68 and 63.15% in *T. parviflorum* and *T. tenuifolium,* respectively. The high content of linoleic acid and palmitic acid may be primarily responsible for significant anti-inflammatory activity. The present study confirms the anti-inflammatory activity of *T. parviflorum* and *T. tenuifolium*.

PB48 Imupret® inhibits respiratory syncytial virus replication and displays *in vitro* and *in vivo* immunomodulatory properties

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Imupret® drops are an ethanolic-aqueous extract of seven different plants (Althaeae radix, Matricariae flos, Equiseti herba, Juglandis folium, Millefolii herba, Quercus cortex, Taraxaci herba). They are used for treatment of recurrent and chronic infections of the respiratory tract, especially tonsillitis. We investigated the antiviral and immunomodulatory potential of Imupret® drops both *in vitro* and *in vivo*. Imupret® inhibited *in vitro* respiratory syncytial virus (RSV) replication (EC_{50}= 6.8 µg/ml) as determined by the plaque reduction assay in MDCK cells. Imupret® increased the percentage of active phagocytic cells (e.g. macrophages) from tonsils of patients with chronic tonsillitis when incubated for 1 h with 1:50 and 1:500 dilutions of Imupret®, suggesting activation of antibacterial immunity. Imupret® also increased the number of CD56+ natural killer cells, important effectors of antiviral immunity. In addition, Imupret® significantly induced antibody-dependent cellular cytotoxicity (25 ± 10% lytic activity in cells treated with 1:500 dilution *vs.* 10 ± 3% in control cells). It also induced the release of IFN-α and IFN-γ. Modulation of cytolytic activity was confirmed *in vivo* by studying the influence of Imupret® on the immune system of healthy, antigen-challenged Wistar rats. Imupret® significantly increased cytolytic activity of splenocytes from rats treated with 0.3x the human equivalent dose during 5 days (55 ± 16% lytic activity in treated *vs.* 26 ± 10% in control animals). In addition, the number of antigen-specific antibody producing cells in the spleen increased significantly (164 ± 25 plaque forming cells/10^6 splenocytes in treated *vs.* 101 ± 16/10^6 cells in control animals). In conclusion, the results show that Imupret® drops inhibit replication of RSV and modulate immune functions relevant for combating bacterial or viral infections which are beneficial for patients suffering from tonsillitis, an infectious disease of mixed viral and bacterial etiology.

PB49 Effects of phenolic glycosides from *Curculigo orchioides* on bone and reproductive tissue in ovariectomized rats

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Phenolic glycosides from *Curculigo orchioides* (COP) have been proven to have estrogen-like and anti-osteoporotic activity [1], and are used for the treatment of osteoporosis and age-associated diseases. The aim of this study was to investigate the effects of COP on bone and reproductive tissue, and to clarify the benefits or risks in the prevention and treatment of osteoporosis. Our results indicated that administration of COP at dose of 6, 18, and 54 mg/kg significantly increased the bone mineral density by 9.0%, 10.9% and 12.3% respectively, improved percent of trabecular area (a marker of microarchitecture structure of bone tissue) from 7.8% to 9.4%, 12.5% and 14.0%, increased the activity of antioxidant enzymes like superoxide dismutase (SOD) from 178U/ml to 202, 234 and 245U/ml respectively, glutathione peroxidase (GPx) from 806 U/ml to 1156, 1209 and 1223U/ml respectively in serum. Nevertheless, COP treatments did not cause manifested fewer adverse effects on the uterus, mammary gland and vagina compared to estrogen administrations. This may be related to their inhibitory effects on the expression of ER α and PR, leading to an increase the ratio of ERβ to ERα. In conclusion, COP may be as effective as estrogen in preventing ovariectomy-induced bone loss, but with fewer adverse effects on the reproductive tissues.
Reference: [1] Vijayanarayana, K., Rodrigues, R.S., Chandrashekhar, K.S., Subrahmanyam, E.V. Evaluation of estrogenic activity of alcoholic extract of rhizomes of *Curculigo orchioides*. Journal of Ethnopharmacology, 2007, 114, 241 – 245

C. Skin-active natural products

PC1 Antimicrobial and cytotoxicity studies of the methanolic extracts of *Erythrophleum ivorense* leaf and stem bark

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Introduction The antimicrobial activity and cytotoxicity of the methanolic leaf and stem bark extracts of Erythrophleum ivorense A. Chev (Leguminosae) were studied to justify its use in treating microbial infections and wounds of many West African countries, including Ghana [1] as well as confirm its toxicity to several livestock [2]. **Method** Their minimum inhibitory concentrations (MIC) against *Bacillus subtilis* NCTC 10073, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 4853, *Escherichia coli* ATCC 25922 and clinical isolate of *Candida albicans* were determined using the micro-dilution method [3] while their influences on cell viability, proliferation and cytotoxicity of HaCaT keratinocytes were also studied [4]. **Results** The leaf methanolic extracts had MICs of 4, 2, 8, 5 and 4 mg/mL for *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*, respectively while the stem bark extract respectively had 2, 4, 3, 4 and 2 mg/mL against these microbes. Within the tested concentrations (0.1 – 100 μg/mL), both extracts decreased the viability and proliferation of the HaCaT keratinocyte cells but did not significantly increased the release of LDH from the cells, compared to the untreated cells. **Conclusion** The demonstrated antimicrobial and cytotoxic activities justified the objectives and confirmed its ethnomedical uses.
References: [1] Irvine (1961) Woody Plants of Ghana. [2] Loder et al., (1974), Aust. J. Chem. 27:179 – 185. [3] Agyare et al., (2013), Pharm Biol, 51(4):418 – 425. [4] Agyare et al. (2011), Phytomedicine, 18(7):617 – 624.

PC2 *Phyllostachys edulis* leaf extract reduces TNFα-induced release of VEGF and IL-8 in immortalized HaCaT cells

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Phyllostachys edulis Carriere (Poaceae) has received considerable attention in pharmacological research due to their potent antitumor, anti-inflammatory, antimicrobial, and antiulcerogenic activities. In this study, we investigated the antiinflammatory effects of two leaf extracts (young versus old harvested leaves) prepared from *Phyllostachys edulis* on TNFα-induced overproduction of IL-8, VEGF and IL-6 in immortalized human keratinocytes (HaCaT). These cytokines play important roles in various inflammatory skin diseases, such as psoriasis. Bamboo leaves of different maturity and age levels were used since we expected a different phytochemical profile and therefore a different pharmacological activity. Both leaf extracts were prepared by Soxhlet extraction using water as extraction solvent. The amounts of major flavonoids were quantified using a LC-MS/MS method. Isoorientin was detected as the main flavonoid in both extracts with amounts of 1590 mg/kg (old leaves) and 1440 mg/kg (young leaves). The Soxhlet extract prepared from the young bamboo leaves (SEYL) dose dependently (25 – 250 μg/ml) inhibited the release of TNFα-induced IL-8, and VEGF, but not IL-6, in HaCaT cells while the extract prepared from the old leaves (SEOL) had no effect. In addition, isoorientin (ISO; 10 – 100 μM) dose dependently reduced the levels of VEGF, IL-8 and IL-6 in TNFα-treated HaCaT cells, comparable to the positive control hydrocortisone (HC; 10 μM). Cell viability was determined by the MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide) colorimetric assay. SEYL and SEOL up to a concentration of 250 μg/ml as well as ISO and HC (10 – 100 μM, respectively) did not have any toxic effects on HaCaT cells. Taken together, an extract prepared from the young leaves of *Phyllostachys edulis* as well as isoorientin exerted anti-inflammatory effects in TNFα-treated HaCaT cells, suggesting interesting cosmetical and pharmacological applications.

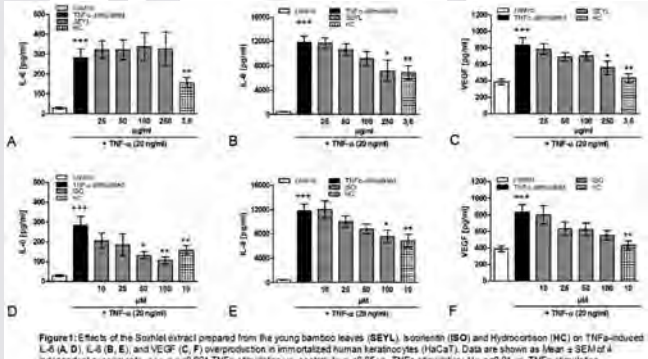

Figure 1: Effects of the Soxhlet extract prepared from the young bamboo leaves (SEYL), isoorientin (ISO) and Hydrocortison (HC) on TNFα-induced IL-8 (A, D), IL-6 (B, E), and VEGF (C, F) overproduction in immortalized human keratinocytes (HaCaT). Data are shown as Mean ± SEM of 4 independent experiments. +++ = p<0.001 TNFα-stimulation vs. control; ** = p <0.05 vs. TNFα-stimulation; ** = p<0.01 vs. TNFα-stimulation

Fig. 1

PC3 Screening of alpine plant extracts as protective agents against UV-induced skin damage

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Incidence of skin cancer has increased in the last few years. High sun protection factors (SPF) in suncare products allow the consumer to expose himself to sunlight for longer periods, but they do not fully protect the skin from harm. Therefore, it is important to develop novel cosmetic formulations which can efficiently fight the negative effects of UV rays, such as production of reactive oxygen species, subsequent cell damages,

and ultimately skin cancer. Alpine plants used in medicine and nutrition represent an attractive source for the development of new products with skin-protective effects. Plants growing at high altitudes have developed natural defense mechanisms including the synthesis of secondary metabolites protecting against exposure to high UV doses. In this context, we screened more than 30 plant extracts for antiradical and anti-inflammatory activities in vitro. Plants collected in the Valais region were extracted with ethanol. Cyclooxygenase-2 inhibitory activity was measured through COX-2 catalyzed prostaglandin synthesis in Mono Mac 6 cells. The formation of 6-keto PGF_{1a} was determined by ELISA. Absence of cytotoxicity was controlled by assessing cell mytochondrial activity (resazurin and MTT bioassays) in Mono Mac 6 cell line. The strongest COX-2 inhibition was observed for the extracts of *Antennaria dioica* (Asteraceae), *Athamanta cretensis* (Apiaceae), *Satureja montana* (Lamiaceae) and *Sisymbrium irio* (Brassicaceae) with 22.7, 26.4, 25.0 and 21.9% inhibition at 100 µg/mL, respectively. In addition, the extracts were screened for free radical scavenging activity against the DPPH radical. In this assay, *Geum montanum* (Rosaceae) (IC_{50} 17 µg/mL) was the most active, followed by *Helianthemum nummularium* (Cistaceae) (IC_{50} 49 µg/mL), *Salix reticulata* (Salicaceae) (IC_{50} 53 µg/mL), and *Satureja montana* (IC_{50} 65 µg/mL). Investigations to identify the active constituents of the most promising candidates are ongoing.

PC4 Influence of the mushroom *Piptoporus betulinus* on human keratinocytes

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Various fungi are known for their biological activities in men and thus are used as medicinal mushrooms. One of these, the basidiomycete *Piptoporus betulinus* (birch polypore), was already used by the ice man (Ötzi), probably as anti-inflammatory agent. Natural compounds and preparations of the fungus sole host genus – *Betula* species – are known for their activity in skin related disorders and are even discussed for their potency in cancer treatment. Triterpenoid compounds like betulin and betulinic acid are assigned to be responsible for these effects [1]. However, there is little knowledge on the natural compounds of the birch polypore and their potential skin protective properties. Therefore, the aim of this study was to investigate the activity of extracts of *Piptoporus betulinus* on a human keratinocyte cell line (HaCaT). Treatment with an ethylacetate extract of *Piptoporus* mycelium resulted in a dose-dependent increase in cell viability and also reduced a serum deprivation induced G_0/G_1 cell cycle arrest. HaCaT cells stressed by UVB broadband irradiation (20 mJ/cm^2) showed a strong cell cycle arrest in G_2/M phase. Incubation with *Piptoporus betulinus* extract after irradiation reduced this effect by 20%. Under the same conditions, the UV-induced DNA damage was diminished. Confirmation of these results by gel free high resolution mass spectrometry based proteome analysis is currently underway. First results indicate an increase of cellular oxidoreductase levels during treatment with *Piptoporus betulinus* extract. On the basis of the presented data a bioassay for the guided fractionation of the ethylacetate extract was established. The study clearly shows that *Piptoporus betulinus* is a promising candidate for both skin cosmetics and active natural compound research. **Reference:** [1] Huyke, C., M. Laszczyk, A. Scheffler, R. Ernst, C.M. Schempp, Treatment of actinic keratoses with birch bark extract: a pilot study. J Dtsch Dermatol Ges 2006; 4: 132 – 136

PC5 Isolation and characterization of wound healing compounds from *Combretum smeathmanii* G. Don

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Combretum smeathmanii G. Don (Combretaceae) is a scandent shrub widely used by traditional healers in Ghana and is indicated for the treatment of wounds and boils [1]. The aim of this project is to investigate skin activity of this plant under *in vitro* conditions on skin cells. Additionally phytochemical characterization and functional testing of secondary metabolites on skin cell lines was to be done. From an ethanol-water (1:1) extract of the leaves, an EtOAc soluble fraction was fractionated on Sephadex-LH20 and prep. HPLC, leading to the isolation of procyanidins as a major compound group. At present epicatechin, procyanidin B2 (epicatechin(4β→8)epicatechin), procyanidin B5 (epicatechin(4β→6)epicatechin), procyanidin C1 (epicatechin(4β→8)epicatechin(4β→8)epicatechin), procyanidin D1 (epicatechin(4β→8)epicatechin(4β→8)epicatechin) and hitherto unidentified oligomeric procyanidins have been isolated. All structures were elucidated by means of ESI-MS and spectroscopic analyses (CD spectra, 1D- and 2D-NMR). Functional studies of extract (at 1 and 10 µg/mL) and isolated compounds from this plant on HaCaT keratinocytes showed significant induction of cellular proliferation in the BrdU incorporation ELISA. These results could justify the use of this plant for wound healing amongs these traditional healers in Ghana. **Reference:** [1] C. Agyare, A. Asase, M. Lechtenberg, M. Niehues, A. Deters, A. Hensel (2009). An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *J. Ethnopharmacol.* 125, 393 – 403.

PC6 Artocarpin attenuates ultraviolet B-induced skin damage in hairless mice by antioxidant and anti-inflammatory effects

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Artocarpin, a prenylated flavonoid isolated from *Artocarpus altilis*, possesses anti-inflammatory and anticancer activities. Because both oxidative stress and inflammation promote the development of ultraviolet B (UVB) irradiation-induced photodamage, the aim of the present study was to evaluate the photoprotective effect of artocarpin on UVB-induced skin damage in hairless mice. Artocarpin at topical doses of either 0.05% or 0.1% showed significant photoprotective effects by decreasing histopathological changes (such as desquamation), epidermal thickening and sunburned cell formation, but the dosage of 0.1% of artocarpin administration was not better than the 0.05% dose. Artocarpin exhibited significant antioxidant activity ($P < 0.05$) by decreasing the levels of reactive oxygen species and lipid peroxidation. In addition, artocarpin significantly decreased the levels of tumor necrosis factor-α and interleukin-1β to down regulate inflammatory proteins, including the synthesis of cytosolic phospholipase A_2 and cyclooxygenase-2 ($P < 0.05$). In conclusion, these data suggest that artocarpin can prevent skin damage from UVB irradiation-induced photodamage in hairless mice, as possibly mediated through antioxidant and anti-inflammation mechanisms. Artocarpin may be useful as a photoprotective agent in medicine and/or cosmetics.

PC7 Efficacy of an herbal multi-component topical TCM therapy for atopic dermatitis (AD) and related comorbid conditions like psoriasis, acne, alopecia, as well as fungal, bacterial, and viral skin infections

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We recently reported the efficacy of a novel TCM therapy – consisting of both oral and topical medications – against AD [1]. Building on these results, we developed improved galenic formulations of two multi-com-

ponent TCM extracts (overall 13 drugs) for purely topical application. All drugs were powdered and extracted in of boiling water for 5 h. Extract 1 was formulated as an herbal soap (1) with anti-inflammatory activity and used for washing the affected areas. For extract 2, three distinct galenic formulations were developed, namely a lotion (2A) with high skin penetrating activity and fast-acting antipruritic effect; a gelatinous jelly (2B) favorable for the treatment of scratching and rupture scars; and a Vaseline based ointment (2C) with long-time activity due to retarded skin penetration and resulting moisturizing and skin protective action for the treatment of dry skin and ulcers.

Tab. 1: TCM plant drugs used in extract 1. extract 2 or both of them, given together with their traditional indications for external use accordmg to Chin Ph; Luo HS, Luo DH Immune Chinese Medians Bering Medical University and China Union Medical University Press. Beijing 1999. and Huang KT. Ding ZZ. Zhao SX Modem Compendium of Materia Medica Chinese Medical Science and Technology Press. Beijing 2001.

Extract	Scientific Name	Plant Part	Japanese - Chinese - Name	Pharmacological Effect (TCM)	TCM Indications (External)	Drug material per litre
2	*Angelica dahurica*	root	トウキ 当帰	improve blood circulation	eczema, fruitbite	10 g
1	*Chrysanthemum indicum*	flowers	キクカ 菊花	anti-inflammatory	eczema, skin infection, cervicitis, mumps	12 g
2	*Citrus x limon*	fruit juice	レモン 檸檬	skin protective, cosmetic, antiseptic, antibiotic, deodorizing	skin protective, fruitbite, cosmetic, insect repellent	5 ml
1	*Coptis chinensis*	rhizome	オウレン 黄連	anti-inflammatory, fistula ulcer, antibacterial	eczema, skin infection, otitis externa, aphthous ulcer	12 g
2	*Dryobalanops aromatica*	resin	ヒュウベン 氷片	anti-inflammatory, antibacterial, analgesic, relieves itching, promotes skin permission of other extracts	pruritus, skin ulcers, eczema, periodontitis, otitis, haemorrhoids	10 g
2	*Glycyrrhiza uralensis*	root	カンゾウ 甘草	anti-inflammatory, treats ulcer, antiallergenic	eczema, psoriasis, skin ulcer	10 g
2	*Isatis tinctoria*	leaves	ダイセイヨウ 大青葉	antipyretic, antibacterial, antiviral	chronic eczema, epidemic mumps	20 g
1	*Paris polyphylla*	rhizome	シチヨウイッシカ 七叶一枝花	anti-inflammatory, dislodgeolent, strongly antibacterial, anti-infective	eczema, bruising, swellings, mumps, mastitis, snake and insect bites	12 g
2	*Polygonum cuspidatum*	rhizome	コジョウ 虎杖	haulk ulcer, haemostatic, antibacterial, anti-infective	suppurative dermatitis, bleeding wounds, scalding, snake bites	20 g
1	*Punica granatum*	pericarp	セキリュウピ 石榴皮	astringent, treats ulcer, skin protective, antimicrobial, antifungal, antiviral	suppurative otitis media, skin ulcer	12 g
1+2	*Scutellaria baicalensis*	root	オウゴン 黄芩	anti-inflammatory, antiallergenic, antibacterial, antiviral	eczema, psoriasis, haemorrhoids	12 g / 20 g
1+2	*Sophora flavescens*	root	クジン 苦参	antiallergenic, antipyretic, antifungal	eczema, skin infection, pruritus vulvae	12 g / 20 g
1	*Stemona sessilifolia*	root	ビャクブコン 百部根	relieves itching, antibacterial, antiviral	skin infection, trichomoniasis, lice	12 g

Both the described fast-acting and long-time effects are extremely important for escaping from the vicious circle of itching, scratching, worsening of the skin eruptions, to worsening of the itching caused by the strong itching feeling of AD. For evaluating the clinical efficacy in the therapy of AD, standardized scores were used for the severities of both AD (clinical severity 0 – 4) and pruritus (pruritus score 0 – 4). Both scores had significantly improved at the end of treatment after two months. Additionally, empirical clinical data on the therapeutic efficacy concerning related comorbid conditions like psoriasis, acne, alopecia, as well as fungal, bacterial, and viral skin infections were collected, demonstration high therapeutic potentials in all these conditions. None of the preparations did display any significant adverse effects, facilitating even prolonged application on new born infants. They can therefore be used in both clinical therapy and self-medication of skin disorders or even in cosmetic applications. **Reference:** [1] Li S et al. Forsch Komplementmed 2013;20:doi 10.1159/000351280

PC8

Evaluation of the wound healing activity of *Buxus sempervirens* L. and *Centaurium erythraea* (L.) Rafn. by using *in vivo* and *in vitro* Methods

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Buxus sempervirens L. (Buxaceae) and *Centaurium erythraea* (L.) Rafn. (Gentianaceae) have been used for wound healing in Turkish traditional medicine. In order to evaluate the wound healing potential of these plants three different extracts were successively prepared from 500 gr of plant materials by extracting with 4000 ml of *n*-hexane, ethyl acetate and methanol at room temparature for 24 hours. The extracts were evaporated to dryness in vacuo. The healing process was investigated with a circular excision and a linear incision wound model to analyse the wound contraction and tensile strength *in vivo*. The anti-inflammatory potential, which is related to the wound healing activity, was evaluated by an *in vivo* experimental model based on the inhibition of acetic acid-induced increase in capillary permeability assessment. Biochemical and histopathological evaluations were done too. For the antioxidant activity assessment DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was applied. According to the results of the present study, methanol extracts of both *B. sempervirens* and *C. erythraea* displayed remarkable wound healing activity with the significant tensile strength values of 30.4 and 34.7%, respectively on the linear incision wound model. Similarly, methanol extracts demonstrated notable wound healing effect with the 59.5 and 71.5% contraction values, respectively on the circular excision wound model.

PC9

Evaluation of *Caesalpinia peltophoroides* in skin keratinocytes and dermal fibroblasts

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Wound healing is a complex process involving several biological events, and a delay in this process may cause economic and social problems. Herbal compounds are assuming an important role in tissue repair[1] and have been used as wound healing agents[2]. Therefore, the aim of this study was to evaluate the cell viability of keratinocytes (HaCat) and fibroblasts (NHDF) *in vitro* after treatment with *C. peltophoroides* crude extract (CE), using the MTT method. *C. peltophoroides* barks were collected at UEM, Brazil, and extracted using 50% ethanol by turbo-extraction (CE). CE was partitioned with ethyl-acetate (EAF) and water. EAF was fractionated by column chromatography on Sephadex LH-20 using 50% ethanol (FA), 100% ethanol (FB) and 70% acetone (FC). FC was fractionated on MCI-Gel® CHP-20P column by gradient (methanol:water) to yield FC 1 – 4. Fractions were monitored by analytical TLC and by HaCat and NHDF, using untreated control (UC-medium) and positive control (PC-5% FCS). The mitochondrial activity was evaluated by MTT test[3]. The statistical analyses were performed using Statistica® 8.0 (ANOVA) with $P< 0.05$ as the significance criteria. An increased cell viability after treatment with CE and FC was observed in both cell cultures with a high activity at 25 µg/mL. The viability in case of HaCat treated with FC 1 is higher compared to FC at 25 µg/mL. However, the NHDF viability increased after 10 µg/mL (Fig. 1). It was observed that CE of *C. peltophoroides* stimulated *in vitro* cell viability of HaCat and NHDF.

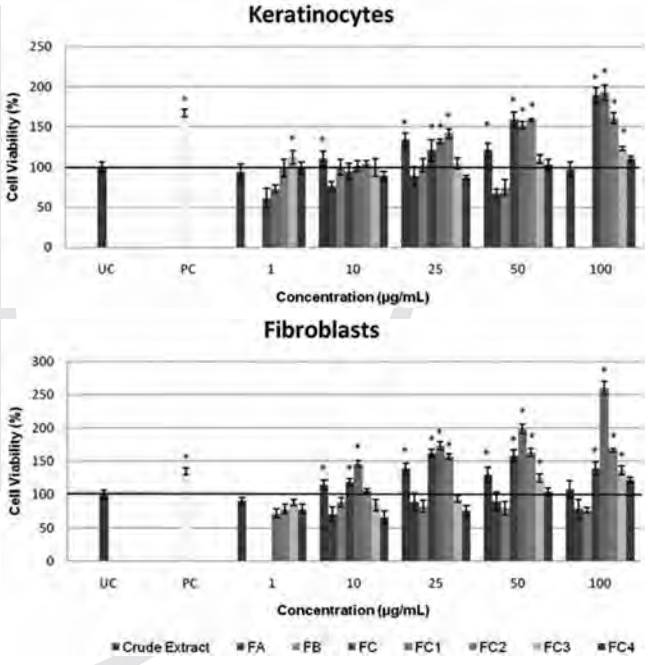

Fig. 1: Cell Viability (%) of keratinocytes and fibroblasts in: crude extract, FA, FB, FC and FC 1 – 4 (1 – 100 µg/mL) (*$P< 0.05$).

Acknowledgements: Fundação Araucária, CNPQ, DAAD. **References:** [1] Sehn, E., Hernandes, L., Franco, S.L., Gonçalves, C.C.M., Baesso, M.L. 2009. *Analytica Chimica Acta*, 115 – 120. [2] Agyare, C., Lechtenberg, M., Deters, A., Petereit, F., Hensel, A. 2011. *Phytomedicine*, 617 – 624. [3] Mosmann, T. 1983. *Journal of Immunological Methods*, 55 – 63.

PC10 Generation of *Leontopodium alpinum* differentiated biomass as a new active biotechnological ingredient

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Leontopodium alpinum, better know as Edelweiss, is a protected asteraceae species only found in mountains above 1800 m of altitude. This delicate plant has received some interest from the cosmetic industry due to its metabolites, and in particular leontopodic acids, which are capable of providing antioxidant and anti-inflammatory benefit for the human skin. The protection of the species along with its specific growing environment is a bottleneck for the large-scale production of active ingredients based on this plant species. To circumvent this issue, we developed several in-vitro propagation methods for the production of undifferentiated (cell suspension cultures) and differentiated biomass (root cultures and leafy-biomass generation) of *Leontopodium alpinum*. Cryopreservation is also implemented to avoid genetic drift of the plant materials and to provide a sustainable production. Quantification of important metabolites, such as leontopodic acid, was performed by HPLC-DAD and the identity of the compounds were confirmed by LC-MS. The impact of plant tissue differentiation is discussed in relation to the choice of biomass to be selected when using *Leontopodium alpinum* extracts in cosmetic creams.

PC11 *In vitro* determination of the skin anti-aging potential of eleven South African plants

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Aging is an inevitable process for all living organisms. In humans, the skin is the most affected tissue. Degradation of the extra cellular matrix leads to an increase in activity of certain dermal enzymes involved in skin-aging including hyaluronidase, elastase and collagenase enzymes directly linked to skin aging. Reactive oxygen species play an important role in many cellular mechanisms including premature skin aging. Skin aging is separable into two forms, chronological aging and photoaging. South Africans are affected by both types because of the harsh sun they are exposed to throughout the year. Small molecules from natural sources have a highly successful track record as pharmaceuticals. The aim of this project is therefore to discover and develop plant-derived small molecules with potential as new and/or improved cosmetic agents. The hyaluronidase, elastase and collagenase inhibition assays were used to assess the skin anti-aging potential of the ethyl acetate and methanol plant extracts. The ABTS-free radical scavenging assay was used to assess the antioxidant activity of the studied plants. Seven extracts showed collagenase inhibitory activity higher (91.21 – 84.70%) than the positive control (EDTA), sixteen showed more than 80% inhibitory activity in the elastase assay with three showing activity comparable to the positive controls elafin (93.09 ± 4.1%) and N-Methoxysuccinyl-Ala-Ala-Pro-Chrolo (91.54 ± 4.1%) and only four extracts showed a percentage inhibition of more than 40% in the hyaluronidase assay. In the antioxidant assay, 10 samples showed good antioxidant activity with an IC_{50} range of 1.99 to 9.45 μg/mL with two of these having a Trolox equivalence of above 0.4. Phytochemical investigation of highly active extracts is underway. The isolated compounds will also be tested in the same biological assays. These preliminary results show that South African plants have an important role to play in the development of naturally derived anti-aging agents.

PC12 Isolation of ellagitannins from *Gei urbani* radix cum rhizoma and determination of extract's anti-inflammatory activity

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Geum urbanum L. is a plant from Rosaceae family. Extracts from its underground parts are traditionally used in external treatment of different skin and mucosa diseases. Despite its position in folk medicine, the external use does not possess any scientific support. The aim of the study was to perform phytochemical examination and to isolate dominating compounds, as well as to determine inhibition of elastase, hyaluronidase and lipoxygenase activity by *Geum urbanum* L. The structures of ellagitannins isolated using column chromatography and preparative HPLC were determined by NMR analysis. Inhibition of hyaluronidase activity was determined by turbidimetric method [1]. Inhibition of LOX activity was determined by spectrophotometric monitoring of linoleic acid oxidation [2]. Inhibition of elastase activity was determined using stimulated neutrophils isolated from venous blood from healthy volunteers by colorimetric method [3]. Two novel ellagic acid derivatives and five known ellagitannins: pedunculagin, stachyurin, casuarinin, gemin G, and dominating gemin A, not previously reported in *Geum urbanum* L. were isolated. Inhibitory values for LOX: extract at 10 μg/mL 48.7 ± 2.2%, gemin A at 10 μM 87.0 ± 2.5% comparing to indomethacin at 50 μg/mL 30.8 ± 2.7%. The IC_{50} value for hyaluronidase inhibition: extract 12.9 ± 1.1 μg/mL and gemin A 5.3 ± 0.6 μM versus reference agent: heparin 62.1 ± 7.51 μg/mL. Elastase release from stimulated neutrophils was inhibited in 30.4 ± 4.8% by aqueous extract at concentration of 10 μg/mL versus quercetin with 44.8 ± 6.6% at 10 μM. These observations can support the traditional use of extracts from *Geum urbanum* L. in external treatment of skin and mucosa pathologies with inflammatory background. **References:** [1] Piwowarski JP, Kiss AK, Kozłowska-Wojciechowska M, J. Ethnopharm. 2011, 137, 937 – 941 [2] Ling SK, Tanaka T, Kuono I, Biol. Pharm. Bull. 2003, 26, 352 – 356 [3] Kiss AK, Bazylko A, Filipek A, et al., Phytomed. 2011, 18, 557 – 560

PC13 Exploitation of plant residues: extraction, separation and characterisation of glycosylceramides

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Ceramides play a significant role in the formation and stability of the skin barrier as major component (35 – 40%) of the *stratum corneum*, the outermost layer of human skin. Additionally they function in signal transduction and cell-cell-recognition processes in the human body, e.g. ceramides may induce apoptosis in human colon-carcinoma cells. Ceramides (Fig. 1) belong to the complex class of sphingolipids and consist of a sphingoid backbone that is acylated with a fatty acid. They occur in all animals, plants and fungi, but also in prokaryotes and viruses.

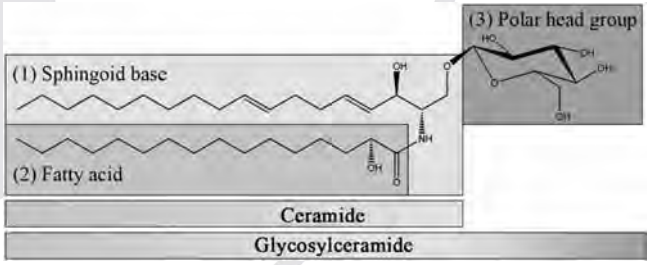

Fig. 1: Representative structure of glycosylceramides in plants

The complex sphingolipid consists of (1) a sphingoid base, (2) a fatty acid and (3) a polar head group-moiety. Glycosylceramide (d18:2 h16:0) is shown as example. Each component may undergo further modifications. Plant and human ceramides vary in their structure. In contrast to human ceramides, those of plant origin are mostly glycosylated (Fig. 1). The various functions of those glycosylceramides in plants are still to be elucidated. The extraction of vegetable wastes seems to be an attractive way to obtain glycosylceramides for further experiments on the role of "phyto"-ceramides in the plants themselves and for possible applica-

tions in health care and therapy. This study concentrates on wheat germ, a by-product of wheat milling. Wheat germ was found before to be a rich source of glycosylceramides. The lipid fraction was extracted quantitatively. Next, a purification method was developed using TLC-guided column chromatography on polar silica gel. Finally, glycosylceramide containing fractions were analysed and structurally characterised by HPLC/APCI-MS.

PC14 Inhibitory effect of *Alpinia katsumadai* on atopic dermatitis-like skin lesions in NC/Nga mice

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Alpinia katsumadai Hayata (AKH) is used traditionally as an herbal medicine in China and Korea. We assessed the effects of AKH extract on the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) in RAW 264.7 cells, thymus- and-activation-regulated chemokine (TARC/CCL17) in HaCaT cells and histamine level in HMC-1 cells. Atopic dermatitis was induced by topical application of house dust mites for 4 weeks, and the protective effects of AKH extract were investigated by measuring the severity of the skin reaction on the back and ears, and plasma levels of immunoglobulin E (IgE) and histamine. AKH extract suppressed the production of NO and PGE_2 in RAW 264.7 cells, TARC in HaCaT cells and histamine in HMC-1 cells in a dose-dependent manner. The severity of dermatitis, including erythema/hemorrhage, edema, erosion and scaling, and plasma levels of IgE and histamine were lower in NC/Nga mice with atopic dermatitis, treated with AKH extract than in untreated mice. AKH extract reduced the histological manifestations of atopic dermatitis-like skin lesions such as erosion, hyperplasia of the epidermis and dermis, and inflammatory cell infiltration on the skin of the back and ear. These results suggest that AKH extract inhibits the development of house dust mite-induced atopic dermatitis in NC/Nga mice. Additionally, we performed simultaneous determination of alpinetin, pinocembrin, and cardamonin in AKH extract using the HPLC-PDA.

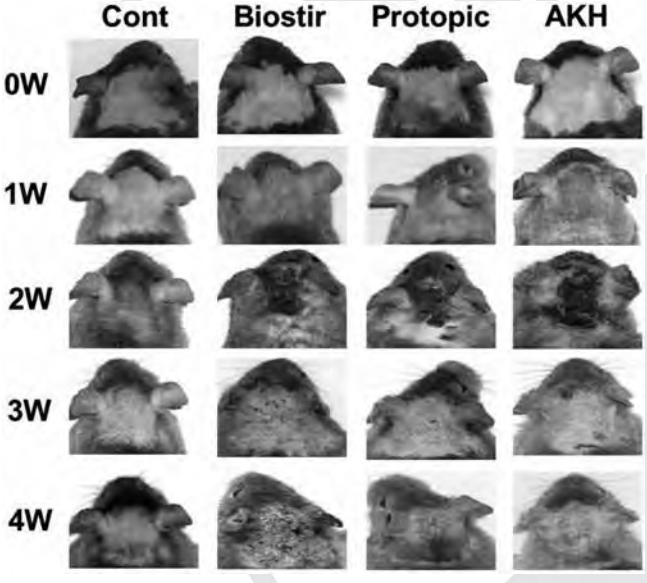

Fig. 1

PC15 Integrated technologies for the discovery and development of cosmeceutical agents from plant biodiversity – NATPROTEC project

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In the framework of the FP7 EU Project "NATPROTEC" an integrated technological platform is proposed for the discovery and development of new agents for the cosmetic industry where safety and *in vitro* biological activity are assessed. The cornerstone of this effort is the Mediterranean and Alpine biodiversity. In this context, literature and empirical knowledge have been leveraged while in parallel, the reverse pharmacognosy concept aiming at the determination of active scaffolds and the rationalized bioprospection is employed. Thus, around 1300 natural compounds were combined from consortium libraries, and computational tools were elaborated to mine putative bioactive compounds targeting histone deacetylase (HDAC) and tyrosinase activities. In parallel, 300 plant species, mainly from the Lamiaceae, Leguminosae, Rosaceae, Boraginaceae and Cistaceae families were selected as potential candidates for their skin protecting effects. The extracts were first tested for their antioxidant (DPPH, ABTS, ORAC) and quinone reductase inducing effects. The hit list of the plants will guide the selection of promising extracts that will be further evaluated for their HDAC activity, anti-ageing (elastase, collagenase and hyaluronidase assays) and anti-hyperpigmenting properties (tyrosinase inhibition). NATPROTEC inspires to integrate this pharmacognosy and reverse pharmacognosy concept and surround them with state-of-the-art technologies aiming at an accelerated research process. **Acknowledgment:** SEVENTH FRAMEWORK PROGRAMME (PEOPLE- Industry-Academia Partnerships and Pathways) Grant agreement no. 286287

PC16 Skin formulation containg *Arrabidaea chica* extract for wound-healing

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Arrabideae chica (Bignoniaceae) popularly known as Carajiru, is a climbing plant widely distributed in South American tropical forest. Leaves of this plant are popularly use in skin diseases. (Devia *et al.* 2002). Wounding has a tremendous impact in healthcare economy; especially chronic wounds represent a major health burden. Recent investigations have shown that plant-derived secondary compounds can serve as new lead compounds for improvement of wound healing. Studies conducted at CPQBA-Unicamp showed that the crude *A. chica* extract has antiulcerogenic and healing action. The cutaneous ulcer lesion index was evaluated on animal experimental model with *Wistar* rats. The ulcers were assessed daily by measuring the contraction area and calculation of the percentage decrease (Jorge et.al. 2008). After 5 days of treatment the animals in the negative control groups (saline) or vehicle (Natrosol,

Carbopol, cream W/O and cream O/W) were stressed presenting redness, exudate and pus, whereas animals treated with *A. chica* extract formulation had a more docile behavior and without pus. After 10 days the formulation evaluated in *in vivo* wound healing experimental models demonstrated 70 to 80% decrease of the ulcerated skin area compared with 37% reduction from the control group. The hidroxiproline content in wound tissue treated with different formulation was observed for the Natrosol ™ gel formulation containing 0,093 $\mu g.mL^{-1}$ of crude extract when compared to other control (0,019 $\mu g.mL^{-1}$) and vehicle (0,028 $\mu g.mL^{-1}$). Histopathological study was showed that well organized fibers. Quality control of the crude extract was accessed by monitoring carajurin (6,50%w/w) content and luteolin (0,50%w/w) content by HPLC with DAD detector.

PC17 The photoprotective and antioxidative properties of the flavonoid luteolin are synergistically augmented by tocopherol and ubiquinone

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Ultraviolet radiation induces DNA damage and oxidative stress which can result in skin inflammation, photoaging and photocarcinogenesis. The flavonoid luteolin that is present in high amounts in the dyers weld, *Reseda luteola,* is one of the most potent antioxidative plant polyphenols that has also ultraviolet-absorbing properties. The aim of this study was to determine whether tocopherol and ubiquinone add synergistic antioxidative values to luteolin. None of the substances showed cytotoxic effects in concentrations from 0.25 to 4 μg/ml. The photoprotective and antioxidant effect of equivalent concentrations of luteolin, tocopherol and ubiquinone and their combination in a ratio of 4:1:4 were studied in solar simulator irradiated human skin fibroblasts. Luteolin had a strong radical scavenging effect at a concentration of 2 μg/ml whereas tocopherol and ubiquinone were not effective at this concentration. None of the substances showed a phototoxic effect and only luteolin had a moderate photoprotective effect at 2 μg/ml. However, the combination of luteolin, tocopherol and ubiquinone exerted a synergistic radical scavenging effect already at a concentration of 0.25 μg/ml and a complete photoprotection at 2 μg/ml. In summary, our findings suggest that the potent antioxidant and photoprotective effect of flavonoids like luteolin may be further increased by the addition of low concentrations of other antioxidants such as tocopherol and ubiquinone.

PC18 Plant-derived inhibitors of human hyaluronidases

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There is a rich tradition in application of natural products in various medical areas: as anti-inflammatory drugs up to drugs in cancer treatment. Hyaluronidases (HAases) appear to be important in many pathophysiological processes, though they are poorly characterized. HAases degrade Hyaluronan (HA), which is a crucial component of the extracellular matrix. Various studies of HAases and HA suggest that an imbalance of HA metabolism has an influence on cell differentiation, skin aging, formation and progression of arthritic diseases and multiple sclerosis and on cancer biology [1]. Concerning their importance in HA metabolism human HAases offer opportunities for drug development. However there is a challenge to obtain catalytically active HAases for *in vitro* studies. All attempts to gain recombinant active Hyaluronidases in *Escherichia coli* were unsuccessful. Furthermore, expression in eukaryotic systems provides only low amounts of protein at high expenses [2]. Via Autodisplay technology we express functional human Hyaluronidases at the surface of *E. coli*. These surface displayed enzymes are then available for investigation and high-throughput screening of inhibitors [3]. In this study we report about Glycyrrhizic acid and curcumin as inhibitors toward human Hyaluronidases and we focus on new plant-derived inhibitors. These substances could serve as starting point leading to the development of new plant drugs against diseases associated with Hyaluronidase activity. **References:** [1] Stern R, Asari AA, Sugahara KN (2006) Hyaluronan fragments: An information-rich system. Eur J of Cell Biology 85: 699 – 715. [2] Hofinger ESA, Bernhardt G, Buschauer A (2007a) Kinetics of Hyal-1 and PH-20 hyaluronidases: Comparison of minimal substrates and analysis of the transglycosylation reaction. Glycobiology 17:963 – 971. [3] Kaeßler A, Olgen S, Jose J (2011) Autodisplay of catalytically active human hyaluronidase hPH-20 and testing of enzyme inhibitors. Eur J of Pharmaceutical Sciences 42:138 – 147.

D. Glycobiology and carbohydrate-based active compounds

PD1 Human galectin binds to multivalent arabinogalactan-protein isolated from aerial parts of *Echinacea purpurea*

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Pressed juices from the aerial parts of *Echinacea purpurea* are used as unspecific immunostimulants, e.g. against common cold. For an arabinogalactan-protein (AGP) from this plant material, immunomodulating activities have been shown *in vitro*, such as activation of the human complement system and binding to human leucocytes (1, 2). The question arises, how high-molecular-weight arabinogalactan-proteins interact with the human immune system when taken orally. One possibility is uptake of AGPs in the area of Peyer's patches of the intestinal immune system (3). Another possible mode of action might be binding of AGPs to galectins present at the brush border membrane of the intestine. Galectins are a family of multifunctional proteins, located in several cells and tissues and characterized by a conserved carbohydrate-binding domain with affinity for β-Gal containing glycoconjugates. Using different ELISA techniques, we proved binding of AGP isolated from aerial parts of *E. purpurea* to human recombinant galectin 3. Galectin 3 is expressed widely in immune cells and also epithelial cells, including gastric and colon mucosa (4). Enhanced binding affinity was found after partial acid hydrolysis of the AGP, which might also happen in the human stomach. **References:** [1] Alban et al. (2002), Planta Med 68: 1118 – 1124 [2] Thude et al. (2006), Phytomed 13: 425 – 427 [3] Taguchi et al. (2004), Carbohydr Res 339: 763 – 770 [4] Takenaka et al. (2004), Glycoconjugate J 19: 543 – 549

PD2 Glucan and galactoglucomannan-like polysaccharides from lichen *Xanthoria parietina*: structural characterisation and effect on murine macrophage cell line RAW264.7

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Lichens are composite organisms of fungal and algal/cyanobacterial symbioses. A wide spread lichen from central Europe, *Xanthoria parietina* was analyzed concerning structural features of polysaccharides. Dried *X. parietina* was defatted and raw polysaccharides were isolated by hot aquoeus extraction and ethanol precipitation. Further fractionation by ion exchange chromatography yielded HI1 and HI2 fractions. GC-MS analysis of partially methylated alditol acetates indicated the presence of (1 – 4)-Glc*p* and (1 – 6)-Glc*p* linked monomers in HI1. Further purification by GPC on Superose CL 6B yielded polysaccharides H1.3 and H2.1 of molecular weight in the range from 200 to 800 kD. H1.3 was found to consist of glucose (< 95%) while H2.1 was composed of galactose (68%), mannose (17%) and minor portion of glucose (5%). ^{1}H-NMR of H1.3 showed signals in the anomeric regions at δ 5.36 and 4.94 which indicated the characteristics of α-Glc*p* configuration, while ^{1}H-NMR of H2.1 at δ 5.19, 5.12 and 4.95 showed α-Glc*p*, α-Man*p* and a dominant component α-Gal*p* residues respectively. Combined data suggested that H1.3 is an α-glucan while H2.1 is characterized as a galactoglucomannan-like structure. The immunomodulatory effect of both compounds have been studied via innate immune cells model. H1.3 and H2.1 exert stimulatory effects on murine macrophage cell line RAW 264.7. by stimulating the macrophages phagocytosis. Both of H1.3 and H2.1 enhanced the macrophage proliferation and phagocytosis of FITC-zymosan to 134% and 124% at 50 μg/mL concentration.

PD3 Antiviral, antioxidant, cytotoxic and hypolipidemic activities of fucoidans isolated from *Sargassum* species

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Sulphated polysaccharides isolated from the brown algae *Sargassum asperifolium*, *Sargassum dentifolium* and *Sargassum linifolium* have been extracted by cold and hot extract. Using *in vitro* models antiviral, antioxidant, cytotoxic and hyperlipidemic activities of isolated polysaccharides were investigated. The polysaccharides were composed by sugars (32 – 53% w/w), sulfur (1.7 – 4.7%), ash (5 – 25%) and the hot extracts contained protein (6 – 10.0%). Fucose and galactose represented the main carbohydrates of isolated polysaccharides. The antiviral activity of isolated polysaccharides were evaluated against HCV (genotypes 1 and 4) using host cells Huh 7.5 and HepG2, respectively and cell viability were assayed by trypan blue dye exclusion method. Also, they were assessed against adenovirus type 40 using host cells Hep-2. Polysaccharide isolated from cold *Sargassum linifolium* showed reduction 60, 16.7 and 60% at non toxic doses 455 µg/ml, 6 µg/ml and 2 mg/ml, respectively. Whereas, hot polysaccharide extract of *S. linifolium* showed reduction 30, 10 and 60% at non toxic dose 0.5 µg/ml, 3.12 µg/ml and 2 mg/ml, respectively. The cold polysaccharide extracts from *Sargassum* species had promising cytotoxic activity against HepG2 with ED_{50} (48.3, 51.15, 61.50 µg/mL). Furthermore, the isolated polysaccharides showed scavenging ability in concentration-dependent manner against DPPH (38 – 64%) and nitric oxide (42.9 – 79.9%) at concentrations 10 – 1000 µg/ml. On the other hand, fucoidan isolated from *Sargassum asperifolium* exhibited hypolipidemic effect which were assayed colorimetric method using (HMG-CoA) reductase and compared with fluvastatin as reference drug. Finally, the results obtained indicating that sulphated polysaccharides isolated from *Sargassum* species might become increasingly important in drug development for treatment hepatic disease. **Acknowledgement:** The financial support for this study by National Research Centre fund (No:9080104) is gratefully acknowledged.

PD4 Anti-HCV, antioxidant, cytotoxic and hypolipidemic activities of water soluble polysaccharides of *Spirulina platensis*

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The microalga *Spirulina platensis* contains 11.09% w/w of total polysaccharides. The water soluble polysaccharides, obtained by cold and hot water extract method, yielded 4.45 and 3.37% w/w, respectively. The total sugar content of cold and hot polysaccharide extracts were 67.29 and 64.66%, respectively. Furthermore according to GC analysis twelve and eleven sugars could be identified in cold and hot extracts, respectively. Glucose, galactose and mannose are predominant sugars in both extracts. The cold and hot water extracts significantly reduced the replication of Hepatitis C Virus (genotype 4) as well as scavenging ability against nitric oxide in concentration-dependent manner. Moreover, the hot extract exhibited cytotoxic activity against HepG2 (human cell line) while the cold extract showed hypolipidemic activity.

PD5 Antiadhesive glycoconjugates from immature Okra fruits against *Helicobacter pylori*

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Helicobacter pylori is a gram negative bacterium which colonizes the human stomach. It is responsible for several gastrointestinal diseases such as gastritis, ulcers, and gastric cancer. The WHO has classified *H. pylori* as a class I carcinogen. Due to increasing resistance to antibiotics the treatment of *H. pylori* infection is faced with severe problems. The adhesion of *H. pylori* to the gastric mucosa can be seen as the first and most important step of the development of its pathogenicity. Thus, the prevention of adhesion by natural compounds can be seen as a new cytoprotective strategy against *H. pylori*. Previous studies indicated that several bacterial adhesins such as BabA and SabA are responsible for the specific attachment to the host cells. Recently it has been shown that an aqueous extract from Okra fruits (*Abelmoschus esculentus*), traditionally used in Asia and Africa for gastritits, inhibits the adhesion of *H. pylori* to human gastric mucosa. This antiadhesive effect is supposed to be related to glycosylated proteins and polysaccharides with a pectin-like rhamnogalacturonan structure (1). The antiadhesive effect of a dialyzed water extract of immature okra fruits against *H. pylori* was proven in an *in vitro* flow cytometric assay by using fluorescence activated cell sorting. The antiadhesive effect was concentration-dependent in the range of 0.2 to 2,7 mg/mL. A concentration of 2,7 mg/mL results in 70% inhibition of adhesion. Protein depletion from this extract by both heat and acid decreases the antiadhesive effect about 20%. This indicates a cumulative effect of both polysaccharides and proteins. Sequential extraction of different polysaccharide classes (pectins, hemicelluloses, etc.) from okra fruits indicated galactoxyloglucan to exert highest antiadhesive effects against bacterial attachment of *H. pylori* (inhibition of 95% for 1,5 mg/mL galactoxyloglycan). **Reference:** [1] Lengsfeld C, Titgemeyer F, Faller G, Hensel A. (2004) J. Agricul. Food Chem. 52, 1495 – 1503

PD6 Polysaccharides from leaves of *Stevia rebaudiana* (Bertoni) and anti-herpesvirus properties

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Herpes simplex virus type 1 (HSV-1) is an enveloped DNA virus that is able to establish latent infection. The prevalence of infection is more than 80% among adults, typically causing oral and eye lesions and even life-threatening disease in immunocompromised individuals. The drugs used to treat HSV-1, including viral DNA polymerase inhibitors, are not always effective or well-tolerated by patients, increasing cases of viral resistance to the drugs. Natural compounds, such as polysaccharides, are being investigated as potential drugs to treat viral infections. Crude aqueous and homogeneous alkaline polysaccharide fractions (i.e., SFW and SSFK-10RM, respectively) from the leaves of *Stevia rebaudiana* (Bertoni) were analyzed to elucidate their anti-HSV-1 action using a plaque reduction assay, cell-cell virus spread analysis, and Western blot of viral glycoprotein expression. The fractions were active in the adsorption and viral penetration phases, with an EC_{50} of 14 and 22 µg/mL for SSFK-10RM and SFW, respectively. Both polysaccharides neutralized viral particles. The assessment of cell-cell virus spread demonstrated that 50 µg/mL of SSFK-10RM and SFW inhibited the lateral spread of infection by 40% and 70%, respectively. Both fractions inhibited the lateral spread of infection by 100% when tested at a concentration of 100 µg/mL. A partial reduction of the expression of viral glycoproteins gB, gC, and gD was observed during the adsorption phase and after virus penetration in the cell. Complete inhibition was observed when the cells were treated during all stages of infection. Our results suggest that *S. rebaudiana* polysaccharides are able to both inhibit the early steps of the infection and exert significant antiviral activity after virus entry into the cell by an unknown mechanism. Thus, *S. rebaudiana* polysaccharides are promising for future research that seeks to treat infections caused by HSV-1 *in vivo*. **Acknowledgements:** CNPq, FINEP, CAPES, Fundação Araucária.

PD7

Anti-inflammatory activity of *Ageratum conyzoides* L. leaves

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The crude extract (the air dried powdered leaves of *A. conyzoides* was extracted by maceration in 70% ethyl alcohol) as well as the successive extracts of the air dried powdered *Ageratum conyzoides* leaves (using petroleum ether (60 – 80 °C), diethyl ether, chloroform, ethyl acetate and methanol respectively) have been evaluated for their anti-inflammatory and chemical constitution. All extracts showed high significant anti-inflammatory activities against carrageenan induce rat paw edema. Quantitative and qualitative estimation of the total flavonoid, steroidal, triterpenoidal, protein and carbohydrate contents of the crude extract have been determined; thus, the protein content of the crude extract was carried out by micro Kjeldahl method. The analysis of amino acids was accomplished using amino-acid analyzerThe mucilage of crude extract was isolated, identified by using HPLC. The free and glycosidal flavonoids of ethyl acetate extract were isolated. Total flavonoidal content of ethyl acetate extract was determined using aluminium chloride colorimetric technique. Quantitative estimation of the total flavonoidal, steroidal, triterpenoidal, protein and carbohydrate contents in the crude extract resulted in 2.52, 1.75, 2.56, 2.91 and 1.80% w/w of dried leaves, respectively. The crude extract significantly inhibited rats paw edema induced by carrageenan by 103.08% and also caused decrease in the IL-6 content by 90.96% relative to indomethacin as a reference drug. The successive extracts of *Ageratum conyzoides* L. leaves had a significant inhibition of the edema formation and significantly decreased of the interleukin-6 content. The isolated flavonoidal fractions and the isolated compounds were evaluated for anti-inflammatory activity. The glycosidal flavonoid fractions proved to have greater anti-inflammatory effect than the isolated compounds.

E. Ethnopharmacology of African medicine

PE1

Evaluation of ethnomedical claims: Antiplasmodial and anti-hyperglycaemic activities of *Gongronema latifolium* root and stem

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The combined root and stem of *Gongronema latifolium* Benth & Hook (Asclepiadaceae) is used in Africa for malaria and diabetes and its leaves as spice. Activity-directed fractionation to identify the active constituent(s) and justify these ethnomedicinal uses in Nigeria was done. Combined root and stem methanolic extract (**GL**), its vacuum liquid (VLC) and column chromatographic (CC) fractions were evaluated for antiplasmodial activities using clinical isolates of *Plasmodium falciparum*- and *P. yoelii nigeriensis*-infected mice, and chloroquine-sensitive (D6) and chloroquine-resistant (W2) *P. falciparum* clones. Their blood glucose reducing and insulinotropic potentials were determined using glucose loaded rats and INS- 1 cells, respectively. Vacuum liquid chromatography of **GL** gave A_1 as the most active VLC fraction while repeated CC of A_1 gave C_1 as the most active CC fraction. Repeated thin layer chromatography of C_1 yielded a 1:1 mixture of α-amyrin and β-amyrin cinnamates (*1a/1b*), lupenyl cinnamate (*2*), lupenyl acetate (*3*) that were characterised using various spectroscopic experiments of IR, NMR and MS. The isolates were also tested against D6 and W2 clones and their *in vitro* insulinotropic abilities were similarly assessed, compared to chloroquine, artemisinin and glibenclamide (positive controls). Lupenyl acetate with IC_{50} 0.033, 0.041 nmol/l against the D6 and W2 clones, respectively was the most active antiplasmodial constituent while 1:1 mixture of *1a/1b*, with comparable high insulin release values as E_1 and glibenclamide, was the most active antidiabetic constituent. These synergistically acting compounds justified the ethnomedical uses of *G. latifolium*.

Tab. 1: Effects of extract, fractions and isolates of *Gongronema latifolium* stem and root

Extract Traction Isolates Compounds	Insulin release (% of glucose effect at 5.6 mM)	
	10 µg/ml	**100 µg/ml**
Glucose (3.0 mM. sub-stimulatory concentration)	62.8 ± 5.9*	62.8 ± 5.9*
Glucose (5.6 mM. negative control)	100.0	100.0
+ MeOH extract (GL)	117.6 ± 6.9	150.7 ± 9.5*
+ Fraction A_1	119.9 ± 9.0	113.7 ± 12.6
+ Fraction A_2	114.4 ± 6.5	133.7 ± 5.4*
+ Fraction A_3	120.5 ± 5.4*	175.9 ± 6.8*
+ Fraction A_4	84.82 ± 7.6	106.9 ± 6.4
+ Fraction A_5	112.0 ± 11.3	127.1 ± 4.6*
+ Fraction A_6	130.2 ± 3.6*	173.8 ± 7.4*
+ C_1	134.6 ± 9.0	176.7 ± 7.5*
+ Y	120.5 ± 6.9	123.0 ± 8.3
+ *1a/1b*	110.0 ± 7.0	178.5 ± 8.5*
+ *2*	133.2 ± 5.3*	148.6 ± 6.3*
+ Z	122.9 ± 2.1*	152.5 ± 3.2*
+ *3*	103.1 ± 5.0	152.8 ± 7.3*
+ Glibenclamide (positive control)	1.0	173.2 ± 6.5*

on glucose-mediated insulin release from INS-1 cells.
GL: Methanolic extract of *Gongronema latifolium* stem and root: **A_1, A_2, A_3, A_4, A_5** and **A_6**: VLC fractions of
GL: C_1: CC subtraction of **A_1**: **Y** and **Z** unidentified isolated titerpenoids; *1a/1b*: 1:1 mixture of α-(*1a*) and β-amyrin (*1b*) cinnamates; *2*: lupenyl cinnamate; *3*: lupenyl acetate. N ± 6 – 8. *: p < 0.5 vs. 5.6 mM of glucose.

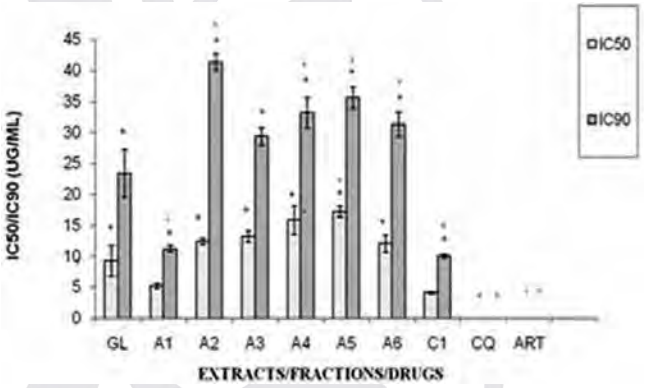

Fig. 1: IC_{50} and IC_{90} of *Gongronema latifolium* stem and root methanolic extract and its fractions against *Plasmodium falciparum* clinical isolates. GL: methanolic extract; A_1, A_2, A_3, A_4, A_5, A_6: VLC fractions;. CQ: chloroquine; ART: artesunate; C_1: CC fraction. †: p < 0.05 comparison against GL; *: p < 0.05 comparison against CQ or ART, using one way analysis of variance followed by Bonferroni post-hoc test N = 6 – 8.

PE2

Antihyperglycaemic and Anti-oxidant Activities of *Eugenia uniflora* Leaf: Evaluation of Ethnomedical Claims IV

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Eugenia uniflora leaf is used ethnomedically in some tropical countries, including Nigeria, in the management of diabetes. Its extrapancreatic anti-diabetic and -oxidant activities have been reported while its insulin stimulation is unknown. *In vivo* anti-hyperglycaemic activities of its methanolic extract (**A**) and partition fractions and their *in vitro* insulin releasing abilities, using glucose loaded rats and INS-1 cells, respectively were investigated. Their anti-oxidant properties as well as *in vivo* insulin stimulatory effects of n-butanol (B_4) and aqueous (B_5) fractions were also studied. The VLC subfractions of most active n-hexane (B_1) were similarly tested for anti-hyperglycaemic and -oxidant activities. Extract (200 and 400 mg/kg) gave significant (p < 0.05) time and dose depen-

dent activities in glucose loaded rats, which were higher than that of glibenclamide (**GLIB**, 5 mg/kg). B_1 was the most active and its lowered activity compared to **A**, suggested synergism in the actions of the constituents. The > 150% insulin releases elicited by B_1 and ethylacetate (B_3) fractions from INS-1 cells agreed with their anti-hyperglycaemic and -oxidant effects and suggested a role of antioxidant in the former activity. The plasma insulinotropic activities of B_4 and B_5 confirmed their *in vitro* abilities and established insulin stimulation as an additional mechanism of action. Anti-hyperglycaemic and -oxidant activities of VLC fractions C_6 and C_7 were similar ($p > 0.05$) and higher than those of B_1 and **GLIB**. Subfraction E_3 was the highest for these two activities, and would contain the main insulinotropic constituents. Also, constituents of E_2, E_6 and E_7 should contribute to these activities. Thefore, contribution of the antioxidant activity of the plant to its antihyperglycaemic property was established, insulin release was established as another mechanism of action of the plant while its ethnomedical usage was justified.

Tab. 1: The antihyperglycaemic *Eugenia uniflora* leaf methanolic extract and its successive chromatographic fractions with high anti-hyperglycaemic activities.

TEST Agent	IC_{50} (mg/ml)			
	DPPH	ABTS	FRAP	TAC
A	0.0860 ± 0.004	0.4616 ± 0.002	0.5941 ± 0.098	0.3837 ± 0.014
B_1	0.707 ± 0.088	3.922 ± 0.021	0.320 ± 0.030	1.103 ± 0.069
C_6	1.4436 ± 0.178[a,b]	2.6740 ± 0.464[a,b]	2.0987 ± 0.279	0.4174 ± 0.003
C_7	1.6587 ± 0.016[a,b]	3.8963 ± 0.175[a,b]	3.4409 ± 0.348	0.1752 ± 0.011
E_2	0.5223 ± 0.018[a,b]	1.7239 ± 0.054	2.3119 ± 0.117	1.1784 ± 0.022
E_3	0.6044 ± 0.047[a,b]	1.8507 ± 0.715	1.0269 ± 0.015	1.0315 ± 0.007
E_6	0.9887 ± 0.004[a,b]	0.4402 ± 0.029	3.4403 ± 0.174	0.6689 ± 0.008
E_7	1.2184 ± 0.072[a,b]	3.5776 ± 0.462[a,b]	4.9450 ± 0.975	0.4949 ± 0.003
Vit. C	0.0083 ± 0.000[a,b]		0.6596 ± 0.100	0.5613 ± 0.023
Trolox		0.1438 ± 0.020		

A: methanolic extract of *Eugenia uniflora* leaf. B_1: n-hexane partition fraction of **A**; C_6 and C_7 most active VLC fractions of B_1; E_2, E_3, E_5 and E_7; most active CC sub-fractions of the combined C_6 and C_7; Vit. C: vitamin C (ascorbic acid, positive control). Trolox: Trolox (positive control). [a]: $p < 0.05$ vs. MeOH; [b]: $p < 0.05$ vs. respective positive control.

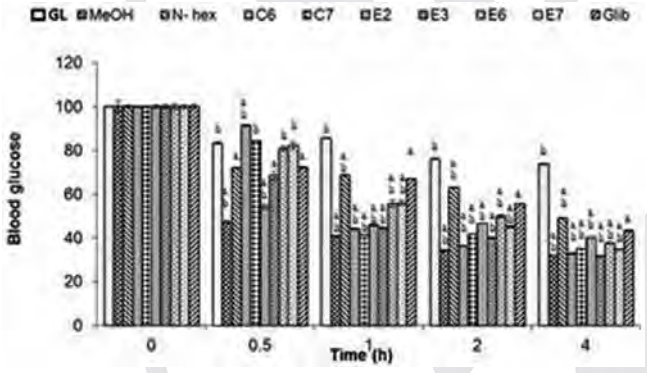

Fig. 1: The antihyperglycaemic *Eugenia uniflora* leaf methanolic extract and its successive highly active chromatographic fractions (200 mg/kg) using glucose loaded rats. Values are given as mean = SEM. N = 5.

PE3

In vivo antiplasmodial activities of four Nigerian medicinal plants

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Infusions and decoctions of *Nauclea latifolia, Artocarpus altilis, Murraya koenigii* and *Enantia chlorantha* are used ethnomedicinally as antimalarial and febrifuge in West and South Africa, especially Nigeria and Cameroon (Madubunyi, 1995; Adjanohoun *et al.*, 1996; Betti, 2002; Ogbonna *et al.*, 2008; Adebayo and Krettli, 2012). Therefore, aqueous-ethanolic extracts of *N. latifolia* root and *A. altilis* stem bark were investigated for prophylactic, chemosuppressive and curative antiplasmodial activities using *Plasmodium berghei berghei* infected mice (Peters, 1967; Ryley and Peters, 1970). The curative activity of *E. chlorantha* was also studied. Using the three models, the activities of all the extracts were significantly ($p < 0.05$) less than those of the positive controls. The standard drug chloroquine gave ED_{50},ED_{90} of 2.19 ± 0.10; 4.29 ± 0.1 and 2.16 ± 0.02; 4.14 ± 0.02 mg/kg for the chemosuppressive and the curative test respectively, while Pyrimethamine gave 0.45 ± 0.11 and 0.91 ± 0.11 mg/kg for the prophylactic test. The order of curative activities was *E. chlorantha = N. latifolia > A. altilis.* The chemosuppressive ED_{50} 235.5 and 279.3 mg/kg and prophylactic ED_{90} values of 455.7 and 356.0 mg/kg for *A. altilis and N. latifolia,* respectively differed significantly ($p < 0.05$). Hence, the extract of *A. altilis had* a higher chemosuppressive activity while *N. latifolia stem* and root had higher prophylactic and curative activities. Therefore, the combinations of *A. altilis* and *N. latifolia,* and *A. altilis*, *E. chlorantha* and *N. latifolia*, as used in herbal decoctions, were evaluated for their antiplasmodial activities in order to have a scientific justification for the ethnomedicinal antimalarial use of these plants. The results may also suggest that their combinations in an herbal potion may be beneficial in preventing, suppressing and curing malaria infection, as is currently obtained in Artemisinin Combination Therapy drugs.

PE4

CNS depressant properties of the crude extract of *Crescentia cujete* in mice

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The study investigated the CNS depressant properties of the crude extract of the pulp of the fruit of Crescentia cujete with a view of establishing the scientific basis of its use in folk medicine (Burkill, 1985). The extract (125 – 1000 mg/kg) was investigated for its effect on novelty induced rearing, grooming, locomotors activity and head dips using open field and hole board test. The anxiolytic effect of the extract was performed using elevated plus maze. The mechanisms of action of the extract were determined with the antagonist; atropine (0.5 mg/kg), yohimbine (1 mg/kg), cyproheptadine (0.5 mg/kg), haloperidol (0.2 mg/kg) and propranolol (0.2 mg/kg). The extract produced dose dependent significant reduction in rearing, grooming, locomotor activity (Table 1) and head dips (28.5 ± 1.6, 24.2 ± 1.5, 20.0 ± 1.4, 18.0 ± 1.4 vs. 39.7 ± 1.7) in mice when compared to control. The inhibitory effect of the extract on rearing and head dips was partially reversed in the presence of atropine showing the involvement of muscarinic receptors in its activity. The other antagonist did not reverse the inhibitory effect of the extract. The extract produced anxiogenic effect on the elevated plus maze. The study concluded that the fruit of Crescentia cujete contain constituents that possess central depressant properties.

Tab. 1: Effect of crude extract of *Crescentia cujete* on novelty induced rearing, grooming and locomotor activity in mice

Treatment	Dose (mg/kg)	NIR/30Min	NIG/30Min	LA/5Min
Control	0.2 ml/20 g	168.4 ± 1.6	40.8 ± 2.0	65.8 ± 1.7
Crescentia	125	146.2 ± 3.7*	33.4 ± 1.2	55.2 ± 0.9
Crescentia	250	92.8 ± 0.9*	28.2 ± 0.5*	49.2 ± 0.9*
Crescentia	500	70.0 ± 1.2*	27.8 ± 0.4*	40.6 ± 0.9*
Crescentia	1000	25.8 ± 0.7*	21.0 ± 1.0*	35.0 ± 0.9*
Diazepam	2.0	7.0 ± 0.7*	11.0 ± 3.1*	25.0 ± 1.2*

Reference: [1] Burkill, H.M. 1985. The useful plants of Tropical West Africa. 2nd Edition. Richmond, UK, Kew Royal Botanical Garden, London 1; 252 – 253 **Keywords:** NIR; Novelty induced rearing; NIG, Novelty induced grooming, LA, Locomotor activity

PE5

Antimicrobial and antioxidant activities of geraniin and aqueous leaf extract of *Phyllanthus muellerianus* (Kuntze) Exell.

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Introduction: *Phyllanthus muellerianus* (Kuntze) Exell. (Family Euphorbiaceae) is used in African ethnomedicine for treatment of microbial

infections and wounds [1]. The aim of the study was to determine antimicrobial and antioxidant properties of aqueous leaf extract (ALE) of *P. muellerianus* and its isolate, geraniin [2]. **Method:** Agar diffusion and broth micro-dilution methods were used to determine the antimicrobial activity of ALE and geraniin against Gram-positive bacteria (*Staphlococcus aureus,* ATCC 25923, *Bacillus subtilis,* NCTC 10073 and clinical strain of *Streptococcus pyogenes*), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and fungal strain (*Candida albicans*). Free radical scavenging activities of ALE and geraniin was determined using DPPH method. **Results:** ALE and geraniin were active against all test microbes with MIC range of 0.08 – 5.0 mg/mL and MBC range of 1.25 – 50 mg/mL (Table 1). The time kill kinetics of geraniin and ALE indicate that mode of action as static within concentration range considered. Antioxidant activities (IC_{50}) of ALE, geraniin and α-tocopherol were 0.123, 1.85 and 0.034 µg/mL respectively. Phytochemical screening of ALE revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids and terpenoids.

Tab. 1: MIC and MBC of aqueous extract of *P. muellerianus,* geraniin and reference drugs (ciprofloxacin and ketoconazole), nd: not determined

Test organisms	*P. muellerianus*		Geraniin		Ciprofloxacin MIC (µg/mL)	Ketoconazole MIC/(µg/mL)
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)		
B. subtilis	1.25	20.00	0.31	5.00	0.10	nd
E. coli	5.00	50.0	1.25	10.00	0.13	nd
P. aeruginosa	0.31	10.00	0.08	2.50	0.25	nd
S. aureus	0.31	5.00	0.16	1.25	0.25	nd
S. pyogenes	0.63	10.00	0.08	2.50	0.10	nd
C. albicans	0.50	5.00	0.16	5.00	nd	5.00

Conclusion: ALE and geraniin possess antimicrobial and antioxidant properties. **References:** [1] Agyare et al. (2009), J. Ethnopharmacol, 125:393 – 403. [2] Agyare et al. (2011), Phytomedicine,18(6):617 – 24.

PE6 Anti-inflammatory and analgesic activity of *Ja*teorhiza macrantha (Menispermaceae)

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Jateorhiza macrantha (Hook. f.) Exell and Mendonça (Menispermaceae) is a common medicinal plant found in tropical Africa. *Jateorhiza macrantha* is used as an anti-hemorrhagic, to combat syphilis and headache [1]. This study was performed to evaluate the analgesic and anti-inflammatory activity of extract. The analgesic activity of the methanolic extract of *Jateorhiza macrantha* was investigated using the acetic acid induced (chemical) and tail-clip (mechanical) models of nociception and the anti-inflammatory activity was investigated using the carrageenan-induced paw oedema in rats. In acetic acid-induced writhing test, the extract at doses 100, 200 and 400 mg/kg significantly ($P < 0.05, 0.01$) reduced the writhing reflex in a dose dependent manner In the application of the metal artery clip unto the tail of animals, the extract caused a significant ($P < 0.05$) dose dependent increase in reaction latency with peak effect (7.0%) inhibition produced at the highest dose of 400 mg/kg. In the carrageenan induced paw oedema test, different doses of the extract produced a dose dependent significant ($P < 0.05, 0.01, 0.001$) inhibition of oedema. The results obtained in this study lend credence to the ethnomedical use of the plant in the management of pain and inflammatory conditions; thus, supporting the development of the biologically active substances as analgesics and anti-inflammatory agents. **Reference:** [1] Burkill, H.M. (1985). The useful plants of West Tropical Africa. Families M – R. Royal Botanic Gardens, Kew, United Kingdom. 2nd Ed. Vol. 4 p. 143.

PE7 Evaluation of anti-diabetic potential of the leaves of *Musanga cecropioides* R. Brown

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The prevalence of diabetes mellitus in the population of African countries is on the increase due to life style changes, therefore; there is a continued need for new and more effective therapy with reduced side effect. Traditionally, *Musanga cecropioides* is used to induce labour, reduce elevated blood pressure and high blood sugar [1]. Previous studies established the hypotensive and oxytocic effects of the water extracts of the leaf and stem bark [2, 3]. This study was carried out to investigate the anti-diabetic potential of the ethanolic leaf extract of *M. cecropioides* using oral glucose tolerance test (OGTT) – (acute hyperglycaemic) and alloxan induced rat model (sub-acute hyperglycaemic). The result obtained in acute hyperglycaemic activity showed a slight anti-diabetic activity of the extract at dose 200 mg/kg though this effect was not significant when compared with control drug – glibenclimide (0.25 mg/kg); while the sub-acute hyperglycaemic activity showed that at days 7, 10 and 14, the extract *M. cecropioides* (200 mg†g) demonstrated a significant ($P < 0.001$; $P < 0.01$; $P < 0.001$) anti-diabetic activity respectively. Oral administration of n-hexane, dichloromethane, ethyl acetate and butanol fractions of *M. cecropioides* at a dose of 200 mg†g significantly lowered ($P < 0.001$) the elevated blood glucose level in the alloxan-induced diabetic rats 5 hr after the administration of the fractions. The summary of the potency of the different fractions of the extract were: Dichloromethane > Ethyl acetate > n-hexane > Butanol, at the administered dose of 200 mg†g for each fraction. It can be concluded that the ethanolic extract of the leaves of *M. cecropioides* given orally at dose (200 mg†g) produced a significant decrease in blood glucose level. **References:** [1] Irvine FR (1961) Woody plants of Ghana. Oxford University Press, London, pp 446 – 447 [2] Kamanyi A, *et. al.* (1992). Phytother. Res. 6(3): 165 – 167. [3] Ayinde, BA. *et. al.* (2006). African J. of Biotech. 5 (14): 1350 – 1354

PE8 An assessment of the toxicity of some plants used locally as aphrodisiacs in Northern Nigeria and their efficacy in enhancing erectile function in rats

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An assesment of the toxicity of five plants, *Lepidium sativum* Linn., *Nauclea latifolia*, *Securidaca longepedunculata*, *Amblygonocarpus andongensis* and *Bridelia africana*, used locally in Northern Nigeria as aphrodisiacs, and the efficacy of the plant extracts in enhancing erectile function in rats were studied. A total of thirty (30) white or albino rats divided into five (5) groups of two (2) males and three (3) females each and a control group were used, making a total of six (6) groups, were used. Fairly uniform conditions of age, weight, temperature, humidity, photoperiod, rat feed and water were maintained before administering the plant extracts in doses of 10, 100 and 1000 mg/kg by the procedure of Lorke (1983), the extracts were found to be practically non-toxic (LD 50 value of 0 mg/kg) according to no observed mortality in each case. The Penile Erection Index (PEI) for each group was determined within a 3 hour period of administration. PEI was calculated by taking the product of Mean episodes of mounting in the rat models and the percentage of rats showing at least one episode. Libido enhancing property was assigned to those samples resulting in high PEI values (from the lowest value of 0.0 to the highest value of 500.0, the standard being 22.0). Extracts of S. longepedunculata exhibited the highest PEI values of 24, 224, 360, 400, 460 and 500. L. sativum had the lowest PEI values. Qualitative analysis of the powdered samples, aqueous and ethanolic extracts based on standard procedures) indicated the presence of saponins, flavonoids, terpenoids, alkaloids, cardiac and steroid glycosides. Flavonoids, however, were not detected in *B. africana* and steroid glycosides were absent in *L. sativum*.

PE9 The effect of *Tetracarpidium conophorum* Mull. Arg. Hutch. & Dalziel (Euphorbiaceae) seed extracts on testicular function in male albino rats

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The effects of extracts of *Tetracarpidium conophorum* Mull. Arg. Hutch and Dalziel (Euphorbiaceae) or African walnut seeds were investigated on testicular function in male albino rats. Four different groups of animals (A – D) were administered with daily oral doses (200 mg/kg body weight) of extracts of n-hexane, chloroform, methanol and water respectively for 21 days. The control group (E) received equivalent volumes of normal saline. The animals were sacrificed after the 14th and 21st days of treatment. Testicular function was determined by serum testosterone levels by ELISA method, testicular weight, semen analysis (1, 2) and histological examination of the testes by H and E stain using Bouins fixative. Results indicated that n-hexane extract caused a significant increase in testosterone levels ($p < 0.05$) in a time dependent manner,

while the methanol and water extracts caused decreases but no change in the chloroform extract-treated group. The body and testicular weights were increased above control values ($p < 0.05$). The superiority of the semen profile was in the order of groups treated with n-hexane>chloroform>water>methanol. Histological examination showed profound increases in spermatogenesis in the n-hexane treated group, while the methanol treated group showed deceases when compared with the control. This study therefore indicated that the n-hexane fraction of *T. conophorum* seeds probably has the ability to increase fertility in male rats and thus lends credence to the ethnobotanical claims in South Eastern Nigeria that ingestion of the seeds boosts male fertility and sexual performance. **References:** [1] Slott, V., Suarez, J. and Perreault, S. (1991). Rat sperm motility analyses: methodologic considerations. Reproduct. Toxicolol 5: 449 – 458. [2] Robb, G. Amman, R. and Killian, G. (1978). Daily sperm production anmd epididymal reserves of pubertal and adultbrats. J. Repro. Fert.54:102 – 107

PE10 Biological activities of *Sutherlandia frutescens* and its future perspectives

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Many indigenous plants have been used for various ailments. *Sutherlandia frutescens* (*SF*) is one of the best known multipurpose indigenous plants of southern Africa, which has been used for many years by traditional healers. Anticancer (against UACC62, MCF7 and HL60 cells) and the *in vitro* cytokine release (interleukins: 1β, 6, 8, 10, 12p70, and TNF) abilities of aqueous and ethanolic extracts of *SF* were examined at the Council for Scientific and Industrial Research, South Africa. For the anticancer screening and cytokine release quantification; Sulforhodamine B (with Etoposide as a positive control) and Cytometric Bead Arrays (with *Echinacea* ethanol extract as a positive control) assays were used, respectively. Here we present the preliminary results and future perspectives of this study. From the different preparations of *SF*; even though the aqueous extracts displayed a decrease in cancer cell viability at higher concentrations; the decrease in cancer cell viability during exposure to the ethanol extracts was greater (on average by about 50%-UACC62, 55%-MCF7 and 85%-HL60; relative to their control). The study showed that an ethanolic extract appeared to recruit TNF and IL8 cytokines to the site of infection upon stimulation with phorbol-12-myristate-13-acetate. The chemical profiles obtained using HPLC-MS provided a good guidance towards the active regions of the ethanol extract; essentially the non-polar compounds present in the ethanol extract contributed to most of the activity observed for this extract. In the future, based on an HPLC activity guided fractionation approach, the research team intends to isolate active compounds from ethanol extracts.

PE11 Determination of Properties Useful for Sourcing Nigerian Phyto-larvicides

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Plant extracts and constituents are better larvicides than the synthetic ones, as they are biodegradable, had low induction of resistance and mammalian toxicity, and comparable activities to the standard drugs. Traditionally, the use of larvicides in the control of vector-borne diseases is unknown. Hence, ethnomedical claims could not assist in sourcing plants that could be phytochemically investigated for their larvicidal constituents. However, there are ethnomedical reports of termite resistance, antimalarial and febrifuge usage. Therefore, methanolic extracts of thirty-six plants, selected based on these claims, were evaluated for activities against 4th-instar larvae of *Aedes aegypti* with the aim of assessing these claims as factors that could be used in sourcing plant larvicides. Sixty-one percent of these plants were significantly active, confirming the usefulness of these properties in choosing plant larvicides. This is the first report of larvicidal activities of the stem barks and leaves of *Blighia sapida* Koenig. (Sapindaceae) and *Baphia nitida* Lodd. (Papilionaceae), leaf and stem of *Costus afer* Ker Gawl. (Costaceae), stem barks of *Artocarpus altilis* Forsberg (Moraceae), *Markhamia tomentosa* K. Schum. (Bignoniaceae) and *Newboldia laevis* (P. Beauv.) Seem (Bignoniaceae), whole plant of *Euphorbia macrophylla* Pax (Euporbiaceae), and *Landolphia owariensis* P. Beuav. (Apocynaceae) leaf. Extracts of *Piper nigrum* L. (Piperaceae), *Abrus precatorius* L. (Fabaceae) and *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae) seeds, *B. sapida* stem bark and *Costus speciosus* (Retz.) Koenig. (Costaceae) root, with LC_{50} 0.01, 0.85, 1.49, 1.71 and 1.47 mg/ml at 48 h, respectively, were the most active, with comparable ($p > 0.05$) or significantly ($p < 0.05$) better activities than Endosulphan (LC_{50} 0.93 mg/ml at 48 h). Hence, they may be used as plant larvicides in the control of dengue and yellow fevers while their investigations may give new larvicidal templates.

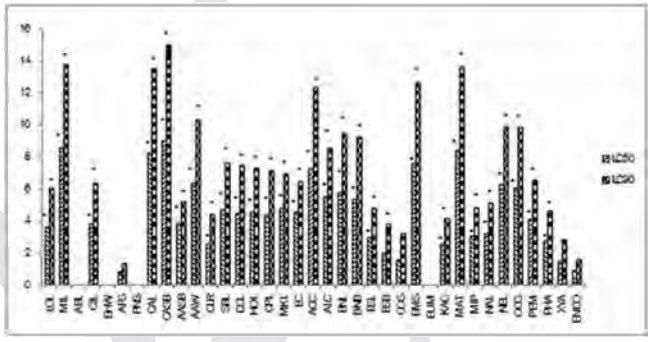

Fig. 1: Effects of methanolic extracts of Nigerian medicinal plants against *Aedes aegypti* at 24 hr.
LOL: Landolphia owariensis leaf; MIL: Mangifera indicia leaf. ABL: Alstonia boonei leaf. CH: Canna indica leaf; Euphorbia heterophylla leaf; APS: Abrus precatorius seed; PNS: Piper nigrum seed; CAL: Costus afer leaf; CASB: Costus afer stem bark; AASB: Artocarpus altilis; AAW: Artocarpus altilis wood; CLR: Curcuma longa rhizome; SBL: Senecio biafrae leaf; DCL: Discoreophyllum cummnisii leaf; HOL: Hoslundia opposita leaf; CPL: Cleistopholis patens leaf; MKL: Murraya koenigii leaf; ECL: Enantia chlorantha leaf; AGC: Ageratum conyzoides whole plant; ALC: Allium cepa bulb. BNL: Baphia nitida leaf; BNB: Baphia nitida bark; BSL: Blighia sapida leaf; BSB: Blighia sapida stem bark; COS: Costus speciosus root; EMS: Emilia sonchifolia whole plant; EUM: Euphorbia macrophylla whole plant; KAC: Kalanchoe crenata leaf; MAT: Markhamia tomentosa stem bark; MIP: Mimosa pudica whole plant; NAL: Nauclea latifolia root; NEL: Newboldia laevis stem bark; OCG: Ocimum gratissimum leaf; PEM: Pentaclethra macrophylla bark; PHA: Phyllanthus amarus whole plant; XYA: Xylopia aethiopica dried seeds; ENDO: Endosulphan (positive control). The larvicidal activities of the extracts were compared with that of Endosulphan (positive control) using one way analysis of variance (ANOVA) followed by Bonferonni post-hoc test. *: $p < 0.05$ significantly different from Endosulphan. N = 6.

PE12 Larvicidal Activities of the Leaves of *Eugenia uniflora* L. (Myrtaceae)

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Eugenia uniflora L. (Myrtaceae) leaves are used ethnomedicinally in Nigeria for diseases such as malaria and in Brazil they are spread on the floor for their insect-repellent activity. Continued search for plant larvicides useful in the control of vector of Dengue fever compelled bioactivity guided fractionation of E. uniflora leaf for lethality against fourth instars larvae of Aedes aegypti. The active methanolic extract (*A*) had larvicidal activity (LC_{50} 2.74 and 2.18 mg/ml at 24 and 48 h) that was comparable ($p > 0.05$) to Endosulphan (LC_{50} 0.93 and 0.90 mg/ml at 24 and 48 h). The n-hexane partition fraction (B_1) with similar activity (LC_{50} 2.58 and 1.87 mg/ml at 24 and 48 h) was the most active partition fraction and should contain the larvicidal constituents. Vacuum liquid chromatography of B_1 and eluting with n-hexane, chloroform and methanol mixtures gave fifth and sixth bulked fractions (C_5 & C_6) with significantly ($p < 0.05$) higher larvicidal activities. Column chromatography of the combined C_5 & C_6, similary eluted as above, yielded 1st and 3rd subfractions (D_1 & D_3) with LC_{50} 1.01 and 1.17 mg/ml at 24 h, respectively and activities that were similar ('$p > 0.05$) to that of Endosulphan

and better than those of C_5 & C_6, only at 24 h. Activities of the D_1 – D_4 subfractions at 48 h were comparable with those of the mother fractions (C_5 & C_6), and Endosulphan. The results established the larvicidal activity of E. uniflora leaf and identified D_1 & D_3 as the most active subfractions that could be further developed as alternative larvicides in the control of Dengue fever, especially among the rural populations of Africa.

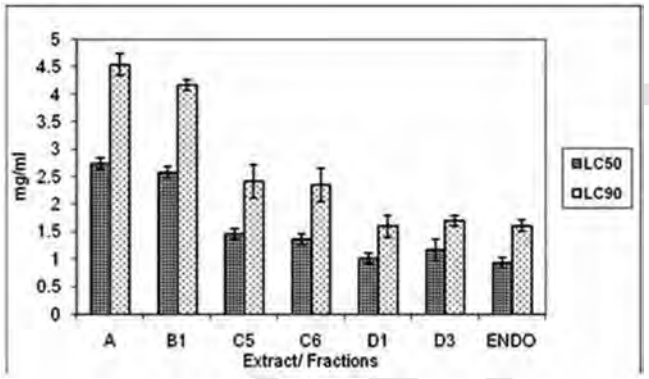

Fig. 1: Larvicidal activities of *Eugenia uniflora* extract and fractions against *Aedes aegypti* at 24 hr.
A: methanolic extract; B_1: n-hexane fraction; C_5: VLC fraction; C_6: VLC fraction; D_1: column fraction; D_3: column fraction; ENDO: Endosulphan (positive control). The larvicidal activities of the extracts and fractions were compared with that of Endosulphan (positive control) using one way analysis of variance (ANOVA) followed by Bonferonni post-hoc test. *: $p < 0.05$ significantly different from Endosulphan. N = 6.

PE13 *Bowiea volubilis* (a highly traded southern African medicinal plant) – Potion, poison or placebo?

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Bowiea volubilis Harv. Ex Hook. f., known as *igibisila* in *isiZulu* is a member of the family Hyacinthaceae, it is widely distributed in the eastern part of South Africa. *B volubilis* bulbs have been utilized in the treatment of various ailments and the plant is a popular medicinal plant and is amongst the top 14% of the most traded plants in South Africa. The aims of the study were to carry out a comparative evaluation of the pharmacological properties of the extracts of *B. volubilis* bulbs grown in the garden against the ones obtained from the *muthi* market and produced *in vitro*. Pharmacological properties evaluated included using antibacterial, antifungal and anti-inflammatory bioassays. The bulb extracts were also evaluated for their possible mutagenic effect. The phytochemical profile of bulbs was also investigated. Results indicated that despite the popularity of the plant in the medicinal trade the supposed uses for the extracts did not necessarily correlate with their pharmacological properties. The antibacterial effect of a number of extracts indicated that the plant does not have significant anti-microbial activity. The plant shows good anti-inflammatory activity which is probably the reason it is regularly prescribed (Masondo, *et al*, 2013). *Bowiea* does contain a number of cardiac glycoside which acquaints to the plant "strength" as a prescribed medicine but may also account for the reported death of some patients due to incorrect dosages. Surprisingly the *in vitro* produced plants appear to have more secondary metabolites in their make-up than outdoor grown ones. In this paper we will discuss the uses and abuse of this highly traded medicinal plant. **Reference:** [1] Masondo, N.A., Ndhlala A.R., Aremu, A.O., Van Staden, J and Finnie, J.F. (2013) A comparison of the pharmacological properties of garden cultivated and *muthi* market-sold *Bowiea volubilis*. *South African Journal of Botany*. 86: 135 – 138

PE14 Antimicrobial ellagitannin-rich extracts and ellagitannins in *Terminalia kaiserana* and *Terminalia sambesiaca*, two African medicinal plants

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Terminalia sambesiaca and *Terminalia kaiserana* are native to East and South African woodlands. Roots, stem bark and leaves are made into hot water decoctions and teas or mixed with maize porridge for treatment of infectious diseases and their symptoms, diarrhea and cough (1). The leaves of *T. sambesiaca* are known to contain saponins (2), but this is the only report on chemistry of this species. *T. kaiserana* has not been studied in this context. Since ellagitannins are known from many species of *Terminalia*, we assumed to find high quantities of them also in *T. kaiserana* and *T. sambesiaca*. Therefore we have analyzed these plant species for their potential as sources of antimicrobial agents, with focus on ellagitannins. Extracts and fractions of stem bark and roots of *T. sambesiaca* and *T. kaiserana*, obtained using solvent partition and Sephadex LH-20 as well as RP-18 and RP-8 column chromatography, were investigated for antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Mycobacterium smegmatis*. Aqueous, butanol and methanol extracts of *T. sambesiaca* roots gave MIC values from 156 to 312 μg/ml against *S. aureus*. UHPLC-MS-TOF results show that the methanol extracts of the roots of *T. sambesiaca* contain moderate concentrations of corilagin, high concentrations of terchebulin and its isomer as well as ellagic acid glycosides. Aqueous and butanol extracts of the roots of *T. kaiserana* gave promising antimicrobial effects against *S. aureus* showing MIC values of 625 μg/ml. An ellagitannin-enriched Lobar RP-8 CC fraction of the roots of *T. kaiserana* effectively inhibited the growth of *S. aureus* with a MIC value of 250 μg/ml. The crude methanol extract of *T. kaiserana* roots is rich in punicalin, terchebulin, punicalagin and ellagic acid rhamnoside. **References:** [1] Chhabra, S.C. et al., 1989. J. of Ethnopharmacol. 25, 339 – 359. [2] Masoko, P. et al., 2005. Afr. J. of Biotechnol. 4 (12), 1425 – 1431.

PE15 Pharmacological evaluation of the anti-diabetic activities of juice formulate (PAMA)

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This study evaluated the anti-diabetic activities of a non commercial plant juice (PAMA) formulated with Picralima nitida (Apocyanaceae), Aloe barbadensis (Liliaceae), Moringa oleifera (Moringaceae) and Allium sativium (Aliaceae). Phytochemical investigations shows that Picralima nitida contain Alkaloids, glycosides, saponins, tannins and phenolic. Aloe barbadensi;-anthraquinone glycosides, resins. polysaccharides, sterols, gelonins. Allium Sativum;- protein, Vitamins, alliin, Ge, Cl, Mn, Zn, Cu, K, Ca, and Fe. Moringa oleifera;- tannins, saponins, flavonoids, cardiac glycosides, alkaloids, steroids and terpenes. 500mls of the extracted plant juice of each plant were collected into a mixer and homogenized for 5mins. The mixture was standardized with sodium methasulphite, blended with 5% honey extract, preserved with 20 ml of Benzoic acid, packaged and stored at 5oC. Total solid, total ash and % acidity was determined (41.28, 23.5and 5.46%). Acute and Chronic toxicity of the juice (LD 50) are 8000 mg/kg and 6000 mg/kg. PAMA (400, 800 and 1600 mg/kg) were administered orally to normoglycemic and diabetic rats, the blood glucose levels monitored with glucomonitor. The result showed that PAMA (1600 mg/kg) caused 46.0% while glyburide (5 mg/kg) caused 53.29% reduction in normoglycemic rats. In hyperglycemic rats, it caused 83.73% and glyburide 70.60% reduction of the blood glucose levels. PAMA maintained reduced blood glucose levels throughout 24 hours duration of study. On chronic (90 days) treatment of hyperglycemic rats, PAMA (1600 mg/kg) caused 89.43%, glyburide (5 mg/kg) caused 80.20% reduction. Hepatotoxicity test showed that it has hepato-protective properties. The hematological Test showed an increased in PCV, WBC and RBC by 14.58%, 10.88% and 12.14% respectively. PAMA has a high hypoglycemic activity which was achieved through reduction of

hepatic overproduction of glucose or increase in glucagon's catabolism, and inhibition of gastric emptying in diabetic conditions.

PE16 Chemical composition and antibacterial activity of essential oils from *Struchium sparganophora* Linn. Ktze Asteraceae

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Struchium sparganophora Linn. Ktze Asteraceae is a culinary herb that is consumed in the Western part of Nigeria and has wide medicinal uses in traditional medicine. The present study was carried out to determine the chemical composition of the oils from its leaf and stem and their antibacterial activity. Essential oils were collected from the leaf and stem of *Struchium sparganophora* by hydrodistillation and analysed by GC and GC-MS. The antimicrobial activity was tested against Gramm negative (G-ve) and Gramm positive (G+ve) microorganisms obtained from the Medical Microbiology Unit, University College Hospital (UCH), Ibadan, Nigeria. Forty-six compounds were identified in the leaf representing 95.3% of the total oil while fifty-five compounds were identified in the stem representing 93.5% of the oil. ß-caryophyllene, Germacrene A, a-humulene and Germacrene D represented the major components in both oils. Antibacterial activity of the oils against certain strains of bacteria showed that the essential oil from the leaf had activity ranging from 9.0 ± 1.0 to 14.3 ± 2.55 mm while the essential oil from the stem had activity ranging from 18.5 ± 2.2 to 20.0 ± 0.0 mm for both G-ve. and G+ve microorganisms respectively. **Keywords**; Essential oils, β-caryophyllene, Germacrene A, antibacterial activity.

PE17 Traditional and indigenous system of medicine in Nigeria

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Medicinal plants are very important to play a key role in human health worldwide. About 80% Africans are depending on traditional use of phytomedicine to cure dreaded diseases like malaria, HIV/AID, sickle cell anemia, yellow fever, diabetes and hypertension. An ethnomedical survey was conducted to document the ethno-medico-botanical knowledge in five villages of western part of Nigeria, especially in Yuroba land of Ogun State-Nigeria. It was surveyed for information on the experience, beliefs and convictions of the residents with respect to safety and efficacy of herbal medicines. This study aimed to establishing resourceful information for both phytochemical and pharmacological studies of the surveyed plants against their ethno-therapeutic claims, which yielded a total of 50 medicinal plants. Plants' habit/habitat of collection showed most of the plants as ubiquitously wild (79%) and herbaceous in nature (66%). Regular users of herbal remedies claimed that they had never experienced any form of poisoning, discomfort or contraindications from use of herbs. It is evident that medicinal plants are continuously being screened for their pharmacological properties and many interesting results with crude extracts have been obtained through the isolation and identification of the active principles. The idea of plant conservation was observed to be lacking. Hence there is a serious threat of decimation and depletion of such plants flora. Regional studies based on epidemiology revealed a record of 34 ailments, which responded as various therapeutic indications for the 50 plants surveyed. Most of the recipes involved a single plant with water and some local liquor as the common extractive solvent, while the mode of administration, dosage-regimens are großly unregulated and unstructured. It is concluded that information of this kind would be of benefit in general health care, ecological control, conservation and research into natural products that leading to drug discovery.

PE18 An ethnobotanical survey of plants commonly used in traditional medicine in Kano, Nigeria

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An ethno botanical survey of plant species used by traditional medicine practitioners in Kano State, North Western Nigeria, was carried out. Data was collected through interviews and administration of questionnaires to 250 people to obtain information on knowledge base of the practitioners, plant parts used in making the preparations and types of prepations, among others. The objectives of the study were to identify the plant species commonly used by traditional medicine practitioners in the study area for the treatment of various ailments and to assess the knowledge base of the traditional healers with respect to the usage of the plant species for treatment of common ailments. The research tool used was the focussed research group which targetted traditional medicine practitioners of the Hausa ethnic group. The investigation revealed that various plant species belonging to twenty-five (25) families are used in the treatment of different ailments in the study area. Majority of these plants (73.2%) are wild species while 18% and 8.2% are wild/cultivated and cultivated species respectively. The most commonly reported plant species were *Azadirachta indica, Gueira senegalensis and Ficus platyphyla.* The validation of botanical taxa by comparing with herbarium collections of the Department of Plant Biology, Bayero University, Kano, Nigeria. The study further revealed that leaves constitute the major (40%) plant part used in making preparations compared to stem (27.2%), root (12.8%) and flower/seeds (8%). Concoctions are the most commonly used type of preparation, while decoctions, infusions, steamed preparations and others constitute 22.8%, 13.8%, 8.8% and 8.8% respectively. Generally the dry form of the plant parts is mostly used and more than one plant species is used in making the preparations as against the use of a single species..

PE19 Ethnobotanical studies of medicinal plants at Biskra Oasis (southern Algeria)

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An ethnobotanical study was conducted at four localities in the region of Biskra, populated by two ethnic groups, Chaouia and Sehari. This survey, conducted in February-March and September-October 2012, identified 64 medicinal plants used in traditional medicine by local people, including 34 collected at Ain Zaatout mainly in the mountains of Aures. - *Agrimonia eupatoria intermedia – Agropyrum repens – Ajuga ìva – Alchemilla arvensis – Alnus glutinosa – Althaea oficinalis – Ammi majus – Ammi visnaga – Artemisia campestris – Artemisia herba-alba – Artemisia vulgaris – Asparagus oficinalis – Asteriscus pygmaeus – Astragabs armafus – Astragalus caprinus – Ballota hirsuta – Bellis annua – Borago oficinalis – Brassica nigra – Calendula arvensis – Calmintha ofsicinulis – Capparis spinosa. – Capsella bursa-pastoris – Carthamus tinctorius – Centclurea calcitrapa – Centuurea qunus – Centaurea nicueensis – Centaurium umbellatum – Ceratonia siliqua – Chenopodium album – Cichorium intybus – Cistus cnespus – Citrullus colocynthis – Clematis ammula – Cleonze arabica – Crataegm oayacanfha – Cupressus sentpervirens – Cynara cardunculus – Cynoglossum cheirifolium – Cynomorium coccineum – Datura stramonium – Diplotaxis harra – Dryopterix filix-mas – Hedera helix – Hertia cheirifolìa. – Hyoscyamus albus. – Hyoscyamus niger – Uypericum perforatum – Jasminun fruticans – Juniperus oxycedrus – Juniperus phoenicea. – Laurus nobilis – Lavandula stoechas – Peganum harmala – Pinus halepensis* -, the ecological distribution and the part of the plant used, the preparation and mode of administration are presented. This work was completed with the identification of field samples and laboratory with the assistance of floras and herbaria available to take traditional folk knowledge to scientific knowledge. Thus, knowledge of medicinal plants, the study area has enabled us to collect as much information on the therapeutic uses practiced by the local population. **Key Words:** Biskra-Oasis – medicinal plants – Chaouia – Sehari – Ain Zaatout

PE20 Ethnobotany and geographical distribution of *Moringa oleifera* Lam (Moringaceae) in Nigeria

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The increase in awareness about the food, medicinal value and other productive potentials of *Moringa oleifera* had elicited interest in research and cultivation in Nigeria. The aim of this study was to obtain ethnobotanical information on the use and local knowledge variation, geographical distribution, and to collect different landraces of *M. oleifera* from different agro ecological regions in Nigeria for further studies (Figure 1). Ethnobotanical data were collected through face to face interviews, semi structured questionnaires and discussions with selected people having knowledge on the plant. The fidelity level (FL %) and use value for different use categories of *M. oleifera* and its parts were estimated. The variation in ethnobotanical knowledge was evaluated by comparing the mean use value among ethnic, gender and age groups using Analysis of Variance (ANOVA). Garmi GPS was used to determine the locations (latitude and longitude) and height in different areas to assess the geographical spread of the species. Seven categories of use (Food, medicine, fodder, fencing, firewood, gum and coagulant) were recorded for *M. oleifera*. Food and medicinal uses showed highest fidelity level while the leaves and the seeds were the plant parts most utilized for the same purposes. There were significant differences among the ethnic and age groups regarding the ethnobotanical use value while there was no significant difference between genders across the various uses. The geographical distribution pattern shows that the *M. oleifera* is well distributed in all ecological zones of Nigeria, well adapted to the varied climatic conditions and gaining unprecedented awareness among the people. Though considered an introduced species, *M. oleifera* has found wide acceptance, recognition and usefulness among the various ethnicities in the studied areas. The sources of introduction, domestication and ethnic differentiation influenced the distribution pattern across the geographical areas.

PE21 Ethnobotany and phytochemical screening of potential anti-malarial plants in South-West Nigeria

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Background: A bio-diverse region such as Nigeria is a promising source of novel anti-malarial lead compounds that is still relatively unexplored. Hence, we carried out an ethnobotanical survey and of the plants used for the treatment of malaria in South-West Nigeria and phytochemical screening of selected promising plants was investigated. **Method:** A semi-structured questionnaire was used to collect information from indigenous people of the South-West Nigeria (Figure 1). The plant samples were authenticated at the Forestry Research Institute of Nigeria. The plants identified as single-used and newly-identified antimalarial herbs were screened for the presence of various phytochemicals quantitatively.

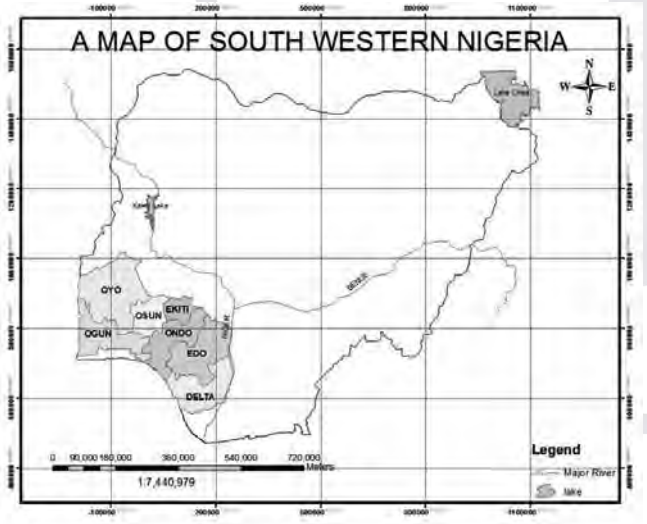

Fig. 1

Results: Of the plants surveyed, *Persea americana* and *Ludwigia peruviana* were identified, for the first time, to be used in the traditional treatment of malaria. *Alstonia congensis*, *Allamanda cathartica*, *Axonopus compressus, Dacryodes edulis*, *Ficus exasperate*, *Bixa orellana*, *Cymbopogon citratus* and *Momordica charantia* were said to be used singly in the traditional herbal therapy of malaria. The phytochemical screening showed the presence of alkaloids, tannins, saponins, flavonoids, steroids, phenols and reducing sugars (Table 1). Cardiac glycosides were found only in *F. exasperate*, *L. peruviana* and *M. charantia* while terpenoids were identified in *B. orellana, F. exasperate, L. peruviana, A. compressus, C. citratus* and *A. cathartica.* **Conclusion:** The identification of these phytochemicals is a step further in validating the traditional claims of the anti-malarial properties of these herbs.

PE22 Assessing the antibacterial activities and toxicity effects of hydroethanolic extract of *Calliandra portoricensis*

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C. portoricensis root is used in the traditional medicine and often administered for a very long period in the treatment of urinary tract infections, dysmenorrhea and other diseases. This study evaluated the antibacterial properties of hydroethanolic root extract of *C. portoricensis* and its safety through acute and subchronic studies in animal. The antibacterial sensitivity was evaluated at 150, 300 and 600 mg/ml concentrations of the extract on *Enterococcus facaelis*, *Streptococcus pneumoniae* (ATCC 49619), *Pseudomonas species* (ATCC 27853). *Escherichia coli*, *Staphylococcus aureus* (ATCC 25923) and *Kleibsiella species* in Agar diffusion method. *E. facaelis* and *S. pneumoniae* only were susceptible with the following diameter of diffusion 15, 12.5 and 11.5 mm for *E. facaelis* and 12.5, 10.0 and 7.5 mm for *S. pneumoniae* while the minimum inhibitory concentrations were 52.2 mg/ml and 6.4 mg/ml respectively. The acute toxicity was evaluated orally in different groups of Swiss albino mice in the dose range of 0.5 g to 20.0 g/kg body weight and observed continuously for 4 h and hourly for the next 12 h and 6hrly for 56hrs (1). Subchronic toxicities were carried out in Wistar rats fed with doses 100, 250 and 500 mg/kg bodyweight of the extract for 30 days and the effects on some biochemical parameters and some tissue histology evaluated. The lethal median dose (LD_{50}) was determined to be 5.0 g/kg body weight. Significant decrease ($p < 0.05$) in low density lipoprotein cholesterol, aspartate aminotransferases, alanine aminotransferases and creatinine was observed while there was increase ($p < 0.01$) in high density lipoprotein cholesterol and total protein levels in all the treated groups. The LD_{50} value obtained implied that the extract was slightly toxic (2) but generally no deleterious effects were observed. **References:** [1] Ogbonnia et al. (2012) Agric Bio J North Am., 3(6), 237 – 246 [2] Klaasen et al. (1995) 8Ed. Mc Graw Hill USA: 13 – 33

PE23 Anti-venom studies on *Olax viridis* and *Syzygium guineense* extracts against *Naja katiensis* venom in rats

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Olax viridis (*Olacaceae*) and *Syzygium guineense* (*Myrtaceae*) are shrubs commonly found in the tropics. They are traditional folkloric medicine for a great number of sicknesses. *Olax viridis* has a wide range of applications in ethnomedicine which include treatment for ulcers, veneral diseases, ringworm, sleeping sickness, diarrhea, fever etc. *Syzygium guineense* has been reported as antidiarrheal agent. Liquid from the bark and roots have been reported to act as a purgative when mixed with water. Both plants have been claimed to have antivenom properties. However, there are no scientific reports on snake venom neutralizing activities of these plants. The plant samples were collected from Olowa in Dekina Local Government Area in Kogi State, Nigeria. The chemicals and reagents used were of analytical grade. Wistar albino rats ♂ weigh-

ing between 180 – 200 g were randomly divided into seven groups of three (3). Groups 1 – 7 received water, normal saline, venom, venom and *Olax viridis*, venom and *Syzygium guineense*, *Olax viridis*, and *Syzygium guineense* respectively. The extracts were administered orally at the dose of 400 mg/kg b.w of rats and one hour later, the venom (0.08mk/kg) was administered. Pulse rate, blood glucose, rectal temperature, plasma cholesterol, triacylglycerol, creatine kinase activity and edema were measured. Significant neutralization of the effects of *Naja katiensis* venom was observed in the groups of rats that received the extracts. Blood glucose, pulse rate, rectal temperature and creatine kinase activity were elevated in the untreated envenomated groups. These results suggest that oral administration of *Olax viridis* and *Syzygium guineense* extracts possess antivenom property, thus, providing the rationale for their use in treatment of sake envenomation.

PE24 Studies on antivenom activity of 50% methanol extract of *Cissus multistriata* against *Naja nigricollis* venom

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The extract of *Cissus multistriata* has been used to cure many diseases. In this work, fifty percent methanol leaf extract of the plant was studied for its antivenom activity against the crude venom of *Naja nigricollis*. Eleven physiologic parameters which include total plasma protein, albumin, creatinine, urea, uric acid, sodium, potassium, chloride, alanine amino transferase, aspartate amino transferase and alkaline phosphatase were measured in the thirty-one albino rats used in the study to monitor the potency or otherwise of the extract against the snake venom. It was discovered that fifty percent methanol leaf extract of *Cissus multistriata* has antivenom effect against *N. nigricollis* venom. Key words: N. nigricollis, Cissus multistriata, antivenom, extract.

PE25 An extract of eastern Nigeria mistletoe, *Loranthus micranthus* Linn modulates dexamethasone-induced insulin resistance and exhibit potent osteogenic activity *in vitro* and in animal experimental model

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Recently, we demonstrated potent *in vitro* osteogenic potentials of different mistletoe extracts and compounds. In the present study, we evaluated the ability of the extract to reverse the deterioration of bone functions occasioned by prolonged therapy with dexamethasone (200 μg/kg i.p) in mature Sprague-Dawley (SD) rats of both sexes. They were treated at doses of 100, 200 or 400 mg/kg daily for 16 – 21 days. The results demonstrated that rats treated with extracts alone had comparable growth rate to the vehicle and positive (Metformin) controls and at the dose of 400 mg/kg, significantly (**$p < 0.01$) reversed severe weight loss caused by dexamethasone. Dexamethasone treatment increased triglycerides levels by 98.75% compared to vehicle group and extract alone ($p < 0.001$) treated but in combination with extracts at 400 mg/kg caused a significant ($p < 0.05$) reduction (44.79%) in amount of circulating triglycerides in serum and preserved serum calcium levels. Data derived from the microarchitectural determination of excised bones showed that the extract (400 mg/kg) in combination with dexamethasone showed better bone mineral density of 1.7532 ± 0.0002*** versus 1.6060 ± 0.0004 for vehicle at the weight-bearing L5 vertebrae. Mistletoe extracts therefore enhanced and preserved bone compactness (quality) of SD rats treated alone with extracts or in combination with 200 μg/kg dexamethasone. The present findings further support the use of mistletoe as an antidiabetic plant with osteogenic potentials. **Reference:** [1] Osadebe, P.O. and Omeje, E.O. (2009). Comparative toxicities and immunomodulatory potentials of five Eastern Nigeria mistletoes, *J. Ethnopharmacol.*, 126:287 – 293.

PE26 Analysis of extracts of eastern Nigeria Mistletoe, *Loranthus micranthus* Linn. (Loranthaceae), parasitic on *Kola acuminata and Garcinia kola* revealed presence of osteogenic compounds

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Osteoporosis is caused by a combination of factors leading to increased risk of fracture. Postmenopausal estrogen deficiency, the major cause in aging women globally contributes to the biggest burden of the disease and presently worsened by the lack of osteogenic therapy. In search of Nigerian plants with osteogenic potentials, we evaluated crude extracts or compounds of the eastern Nigerian mistletoe, a known antidiabetic plant traditionally claimed as a putative panacea for post-menopausal syndromes. Crude methanolic extracts of mistletoe harvested from three host tress; *Kola acuminata*, *Citrus spp* and *Garcinia kola* and five abundant isolated compounds including 3, 4, 5-trimethoxybenzoate (KH), lupeol (LP), 7α, 15β-dihydroxy lupeol palmitate (DLP), friedelin (FRD) and 3-methoxy quercetin (QD) were evaluated for their osteogenic potentials using osteogenic models. Results showed that the crude extracts (0.2 – 1.6 μg/ml concentrations) exhibited significant increase in the alkaline phosphatase activity of osteoblasts compared to control cells, exhibiting EC_{50} values of 52.60-, 2.72- and 637.00 μg/ml respectively for *Kola acuminata*, *Citrus spp* and *Garcinia kola*. None of the extracts had cytotoxicity to osteoblasts at the concentrations tested. The compounds (100pM-100nM concentrations) except FRD produced 3 – 5 folds increase in alkaline phosphatase activity and significantly enhanced mineralization (> 2-fold) of cultured osteoblasts compared to control osteoblasts with very low EC_{50} values (QD-0.001 μM, KH-0.0028 μM, DLP-0.97 μM, LP-2.98 μM, FRD-8.11 μM). All compounds strongly induced the expression of key osteogenic genes including BMP-2 and RUNX2. In conclusion, the crude extract and compounds from the mistletoes species possess potent *in vitro* osteogenic activities and may be developed as safer alternative(s) in the management of diseases where bone loss is the pathology. **Reference:** [1] Omeje, E.O., et al. (2011a), *Phtyo. lett*, – DOI: 10.1016/j.phytol.2011.07.011.

PE27 Anti-diabetic, anti-hyperlipidaemic and anti-oxidant effects of total saponin fractions of *Irvingia gabonensis* stem bark on streptozotocin diabetic female rats

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Our studies have revealed that aqueous stem bark extracts of *Irvingia gabonensis* possess long term (24 weeks) antidiabetic and antihyperlipidaemic effects. In this study we evaluated the short term (28 days) antidiabetic, antihyperlipidaemic and antioxidant effects of *I. gabonensis* total saponin fractions (ITFS). ITSF at 10 and 20 mg/kg body weight treatment caused only a marginal decrease in fasting blood sugar compared to untreated diabetic group. The lowest dose used (10 mg/kg body weight) produced the greatest reductions in serum total cholesterol, triglycerides and LDL-cholesterol, while 10 and 20 mg/kg body weight saponins treated male diabetic rats (TMDR) had significantly ($P < 0.05$) increased HDL-cholesterol concentration compared to untreated diabetic control. Serum and tissue malondialdehyde (MDA) concentration significantly ($P < 0.05$) increased in diabetic control group compared to normal control; treatment with ITSF significantly ($P < 0.05$) reduced the MDA concentration. Serum and tissue superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase (CAT) activities were generally increased in the saponins TMDR compared to untreated diabetic control. This study revealed that the short term antidiabetic effect of ITSF was only marginal, however a substantial antihyperlipidaemic effect was observed in the TMDR. ITSF also showed a noticeable antioxidant effect in the treated rats. We conclude that the phytochemicals found in *I. gabonensis* bark, including saponins, may act together to exert its antidiabetic effect; it is also possible that a longer period of time may be necessary for the anti-diabetic effects of *I. gabonensis* saponins to manifest.

PE28 Long-term anti-diabetic and anti-hyperlipidaemic effects of aqueous stem bark extracts of *Irvingia gabonensis* in streptozotocin-induced diabetic rats

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Our previous studies have shown that aqueous stem bark extracts of *Irvingia gabonensis* possess long term hypoglycaemic effects in normal rabbits. This study was designed to evaluate the long-term anti-diabetic and anti-hyperlipidaemic effects of aqueous stem bark extracts of *I. gabonensis* in streptozotocin-induced diabetic rats. Twenty four Wistar rats in three groups, normal control, diabetic control and *I. gabonensis* treated diabetic rats (TDR) were used for this study. Diabetes was induced in 16 rats by intraperitoneal injection of streptozotocin (STZ) at 65 mg/kg body weight. Upon confirmation of diabetes, the treated diabetic rats were orally (by gavage) given aqueous extracts of *I. gabonensis* bark at 200 mg/kg body weight daily for 24 weeks. Body weight was monitored weekly, while fasting blood sugar (FBS) and serum lipid profile (triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol) were assessed at specific intervals for 24 weeks. *I. gabonensis* reduced the FBS of the treated diabetic rats to normal control levels 2 weeks after the commencement of treatment. The reduction of FBS was sustained till the end of the study (24 weeks). Furthermore, at various stages of monitoring, the extract reduced the STZ-induced elevation of serum triglycerides, total cholesterol and LDL-cholesterol, and significantly ($p < 0.05$) increased the STZ-induced decrease in HDL-cholesterol. Our study concludes that aqueous stem bark extracts of *I. gabonensis* possess profound long-term anti-diabetic and hypolipidaemic effects. These anti-hyperlipidaemic effects as well as the presence of phytochemicals with recognizable anti-oxidant effects will be useful in the treatment of diabetic complications.

PE29 Evaluation of the erythropoietic and anti-sickling properties of *Ficus capensis* leaf extract in the treatment of anaemia

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Ficus capensis is used locally in the treatment of haemolytic and sickle cell anaemia in the south eastern Nigeria. This research sought to ascertain the erythropoietic and anti- sickling effect of the leaf of Ficus capensis. The ethanolic and aqueous extracts of the air- dried and pulverized leaves were obtained using 95% ethanol and distilled water respectively. Phytochemical tests and proximate analysis were carried out on both the dry powdered leaf and the leaf extracts using standard methods and the result obtained showed that the plant contains significant amount of Lipids, Flavonoids, Reducing sugar, Saponins, Tannins, Anthraquinone, Starch, Proteins and glycoside. Antimicrobial screening was done using standard method. Animal study involving 5 groups of 5 rats each was carried out. The first 3 groups were administered oral doses of 50 mg/kg, 100 mg/kg and 200 mg/kg of the extracts, while the last two groups served as negative and standard reference control for 14days, with intermittent withdrawal of blood in days 0, 3, 7 and 14 from the retro-orbital veins for haematological analysis; PCV, RBC, WBC, Hb using Neubau counting chamber. The PCV test showed in increase with 50 mg/kg (44%.- 49%) and 100 mg/kg (45% – 58%) against 45% – 49% in standard reference control and 44% – 45% in Negative control, RBC 6.70 d ± 0.08 to 8.82a ± 0.31, WBC 11.6 d ± 0.44 to18.8c ± 0.60 and Hb,14.60a ± 0.49 to19.9 d ± 0.35. Anti-sickling activity of the plant extract was determined by adopting the Emmel test procedure using blood samples from sickle cell patients, ages13, 26 and 32years. The Anti-sickling test shows inhibition of sickling by the extracts at 32.81% and 36.9% respectively on both SS red blood cell samples from the old patients using concentrations of 50 μg/l and 100 μg/l. The result indicates significantly high erythropoietic action of the plant leaf and anti-sickling properties. This justifies its ethno-medicinal use in the treatment of anaemia and anti-sickling crisis.

PE30 Plants used in Mali against malaria and their content of polysaccharides with immunomodulatory properties

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Most of the traditional healers in Mali that have been interviewed about plants used for the treatment of Malaria say that they do prescribe that the plants mainly should be taken as water extracts. However, most studies on medicinal plants up to the present time have been concentrated on organic solvent extracts and very little work has been performed on aqueous extracts. Thus, the aim of one part of the EU project MUTHI (Multi-disciplinary University Traditional Health Initiative: Building Sustainable Research Capacity on Plants for Better Public Health in Africa) is to focus on water-soluble high-molecular compounds like polysaccharides in plants used against malaria. As most illnesses will involve the immune system it was our theory that the effect on the patients using these plants partly could be due to the effect they have on stimulating the immune system. The plants in focus are those present in the registered product *Malarial* consisting of *Cassia occidentalis*, *Lippia chevalierii* and *Spilanthes oleracea*; *Argemone mexicana, Erythrina senegalensis* and other plants traditionally used against malaria by the healers from different Malian regions have also been included in the study. Results show that the water extracts are rich in polysaccharides, their monosaccharide compositions vary between the plants and indicate the presence of both pectins and glucans. The effect on the immune system was tested primarily with the complement assay, but also on the stimulatory effect of macrophages. The polysaccharides from the Malarial complex showed good activity, so did also those from *Argemone mexicana,* also a plant showed to be effective in the treatment of malaria. Several of the other plants studied did also show promising activities. These results will be discussed in relation to the carbohydrate content.

PE31 *Combretum apiculatum* (Combretaceae): Antibacterial testing of extracts from the leaves and seeds

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Combretum apiculatum (red bush willow) is a small to medium-sized deciduous tree which occurs in various savanna regions. Traditional healers use a steam bath from leaf decoctions to treat abdominal disorders and microbial infections. They also treat inflammation that causes headache and toothache, and conjunctivitis. The objective of this study is the screening of sequential dichloromethane (DCM) and methanol extracts from the leaves, and the methanol extract from the seeds of *Combretum apiculatum* against the *E. faecalis*, *K. pneumoniae*, and *C. neoformans* bacterial strains, and the subsequent isolation, purification, and characterization of any active compound. A sample of powdered leaves (80 g) was extracted sequentially with DCM and MeOH, yielding 13.4 g and 40.1 g of extract, respectively. A sample of the methanol extract (35 g) was suspended in water and partitioned with heptane (Hept), ethyl acetate (EtOAc), and water saturated with butanol (BuOH). The seeds were extracted with methanol to yield 20.3 g of crude extract. The seed extract (17 g) was suspended in water and partitioned with heptane, ethyl acetate, and water saturated with butanol. All of the 11 samples exhibited a noteworthy antimycobacterial effect on at least one of the bacterial strains, with four samples showing activity against all three bacterial strains. Minimum inhibitory concentrations were determined using the INT micro-well method (NCCLS, 2003) [Table 1].

Tab. 1: MIC values expressed in mg/mL

Sample	Diluent	Concentration	*E. faecalis* ATCC 29212	*K. pneumoniae* ATCC 13883	*C. neoformans* ATCC 90112
Seeds MeOH extract	Acet	32 mg/ml	0.13	0.25	Insufficient
Seeds Hept	Acet	5 mg/ml	1.25	0.63	0.48
Seeds EtOAc	Acet	5 mg/ml	0.94	0.32	0.03
Seeds BuOH	Acet	5 mg/ml	> 1.25	0.48	0.02
Seeds H_2O	Acet	5 mg/ml	> 1.25	1.25	0.04
Leaves DCM extract	Acet	32 mg/ml	1.00	> 8.00	0.50
Leaves MeOH extract	DMSO	32 mg/ml	2.00	4.00	1.00
Leaves Hept	Acet	5 mg/ml	> 1.25	> 1.25	0.08
Leaves EtOAc	Acet	5 mg/ml	0.63	0.63	0.32
Leaves BuOH	Acet	5 mg/ml	> 1.25	0.32	0.04
Leaves H_2O	Acet	5 mg/ml	0.63	0.63	0.32

PE32 Microscopic characterization of medicinal plants commonly used in the Hamer region, South-western Ethiopia

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People in Ethiopia have used medicinal plants as a major component of their traditional medical practices for many centuries. Details of medicinal plant application and of healing qualities are transferred from generation to generation mainly by words of mouth. This preservation of knowledge is error-prone, in particular, as plant biodiversity in Ethiopia is high and occasional confusion of species is possible. The prevalence of the use of traditional medicine among the Hamer community, south-western Ethiopia, was obtained by questionnaires which were answered by key informants including eight traditional healers and 1600 household respondents supplemented by eight focus group discussions. A total of 60 medicinal plants were reported with their local names, indication(s), parts used and method of preparations. It was found that the most widely used medicinal plants are *Albizia anthelmintica*, *Aloe otallensis*, *Amaranthus hybridus*, *Carissa spinarum*, *Datura metel*, *Lagenaria siceraria*, *Maytenus senegalensis*, *Moringa stenopetala*, *Salvadora persica* and *Solanum incanum*. More than 20 medicinal plants out of the species reported to be applied medicinally were collected for detailed microscopic analysis. The pictures will help to distinguish species and to perpetuate the traditional Ethiopian plant knowledge. The development of plant monographs with microscopic pictures of commonly used medicinal plants in Ethiopia is part of the DAAD supported project "Welcome to Africa".

PE33 Evaluation of retinoblastoma (Rb) and protein-53 (p53) gene expression levels in breast cancer cell lines (MCF-7) induced with some selected cytotoxic plants

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Many Nigerian Plants have been hypothesized to have anticancer potentials.(1) However, six of such plants were selected to evaluate the effects of their crude, hexane, chloroform, ethylacetate, detanninfied and tannin fractions for brineshrimp lethality assay and the most cytotoxic fractions of each plant were further tested on gene expressions of p53 and retinoblastoma (Rb) in human breast cancer cell line (MCF-7). *Gladiolus psittacinus* (Gps), *Icacina trichantha* (Itr), *Spilanthes filicaulis* (Sfi), *Curculigo pilosa* (Cpi), *Anthocleist adjalonensis* (Adj), and *Tapinanthus bangwensis* (Tba) medicinal plants were selected. Crude extracts of 80% aqueous ethanol macerated plant materials were fractionated into hexane, chloroform and ethylacetate fractions. 5 g each of concentrated crude extracts were dissolved in 20 ml deionised water and 70 ml of the organic solvent (n-hexane) was added and the immiscible mixture was transferred into a separatory funnel. The mixture was allowed to phase separate to aqueous and organic fractions. The aqueous fractions gave detanninfied and tannin fractions. The 36 panel of plant fractions produced from all the plants were used for the study. Hexane fraction of *Spilanthes filicaulis* (Sfi-HF) showed the highest cytotoxic effect (LC50 21.30 µg/ml) on brineshrimps showing a low signal of p53 gene expression but a high intensity of retinoblastoma (Rb) gene expression in MCF-7 cell lines. Crude extract of *Gladiolus psittacinus* (Gps-CE) showed a significant ($P < 0.05$) increase in p53 gene expression in comparison with the control group and a high intensity of Rb gene expression. Results demonstrate the modulatory potentials of Sfi-HF and Gps-CE on p53 and Rb gene expressions in MCF-7 breast cancer cell lines suggesting a possible mode of action of Sfi-HF and Gps-CE amongst a panel of 36 extract fractions. Key words: p53 gene, retinoblastoma (Rb) gene, brineshrimps, cytotoxicity, gene expression. **Reference:** [1] Sowemimo et al 2007, J. Ethnopharmacol 113(3) 427 – 32.

PE34 Antibacterial effects of stem bark and wood extracts of African medicinal plants *Terminalia laxiflora* and *Terminalia brownii*

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Terminalia laxiflora and *Terminalia brownii* are used in African traditional medicine for treatment of infectious diseases and their symptoms, such as venereal diseases, cough, inflammations, eye diseases and skin disorders (1). *T. laxiflora* has not been studied before for antibacterial activity, and to the best of our knowledge there exists only one earlier study on antibacterial effects of *T. brownii* (2). Because of this we have evaluated these plant species for their potential as sources of antibacterial agents. Extracts of stem wood and bark of *T. laxiflora* and *T. brownii*, obtained using sequential extraction and solvent partition, were investigated for their antibacterial activities against the human pathogenic *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus* and *Pseudomonas aeruginosa*. The aqueous and ethyl acetate extracts of all the investigated plants gave in general the best antimicrobial effects, with the exception of a chloroform extract of *T. laxiflora* stem wood, which gave outstanding effects against *P. aeruginosa* (Figure 1). Ethyl acetate extracts of the stem bark of *T. laxiflora* gave MIC values of 39 µg/ml against *P. aerginosa*, and were more effective than gentamycin (MIC 62 µg/ml) against this bacterial strain. Promising MIC values of 156, 312 and 625 µg/ml of an ethyl acetate extract of the stem wood of *T. laxiflora* were recorded against *P. aeruginosa*, *M. luteus* and *S. aureus,* respectively. Low MIC values of 156 µg/ml were also recorded for ethyl acetate and aqueous extracts of the stem wood of *T. brownii* against *M. luteus*.

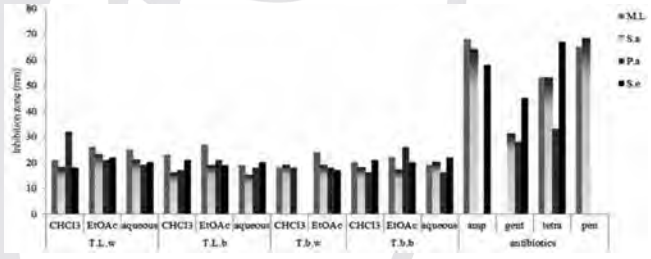

Fig. 1: Antibacterial effects of stem bark and wood extracts of *T. laxiflora* and *T. Brownii*
T.L.w, *T laxiflora* stem wood; T.L.b, *T laxiflora* stem bark; T.b.w, *T brownii* stem wood; T.b.b, *T brownii* stem bark: $CHCl_3$, chloroform extract; EtOAc, ethyl acetate extract; aqueous, aqueous extract; amp, ampicillin; gent, gentamicin; tetra, tetracycline; pen, penicillin; *M.l. Micrococcus luteus*; *S.a*, *Staphylococcus aureus*; *P.a*, *Pseudomonas aeruginosa*; *S.e*, *Staphylococcus epidermidis*. Results as diameter of inhibition zones in mm (n = 4).

References: [1] Neuwinger, H.D., 2000. African traditional medicine. Press Stuttgart, Germany. [2] Mbwambo, Z. H. et al, 2007. Complementary and Alternative Medicine 7 (9), 1 – 5.

PE35 Ethnopharmacological Use of Wild Medicinal Plants in Western Desert and Oasis Region, Egypt

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Background: "Back to Nature" is our Moto for solutions to human health problems, both within as well as outside Egypt, has important

implications for local, especially tribal communities. **Materials and methods:** The survey was based on qualitative and quantitative research on both the collection and analysis of data, both social and cultural beside ethnographic literature through numerous field trips that gives a very rich background to different life aspects of inhabitants of the Egyptian Western Desert and Oases. The ethno botanical survey was carried out on the Wahateya (inhabitants of the Oases in Egypt). The areas of investigation were Bahariya, Farafra, Dakhla, Kharga, Siwa, & Wadi-el-Natrun Oases. Visiting private farms and households interviews were conducted with men, women and children. **Results:** A total of more than 250 plant species were recorded, of these 53 species were considered to be of medicinal value as for example: *Hyoscyamus muticus* L.; *Citrullus colocynthis* (L.) Schrad.; *Calotropis procera* (Aiton.) W.T.; *Balanites aegyptiaca* (L.) Delile.; *Portulaca oleracea* L. and *Artemisia monosperma* Delile;. Representative herbarium specimens (about 100) and available seeds (about 30) were collected. Botanical and ecological surveys as well as phytochemical and biological surveyes were carried out. The Wahateya were found to possess a highly knowledgeable recognition about plants especially those of the medicinal value. This knowledge is passed from one generation to another. **Conclusions:** Conservation and sustainable use of wild medicinal plants in the studied area is highly important and women play an important role in this sector. Development of this sector is highly recommended. **References:** [1] Ross, I. A. (1999). Medicinal Plants of the World. Chemical Constituents, Traditional and Modern Medicinal Uses. Humana Press, Totowa, New Jersy. [2] Boulos, L. (2000). "Flora of Egypt", volumes 1 – 3, printed by Al Hadara Publishing, Cairo, Egypt.

PE36 Antiplasmodial activity of some plants used by Zulu traditional healers and some of triterpenes isolated from the plants

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Mimusops caffra E. Mey. ex A.DC., *Mimusops obtusifolia*, Lam (both members of *Sapotaceae* family) and *Hpoxis colchicifolia*, Bak are used by traditional healers in Zululand to manage malaria. We have investigated the anti-plasmodial activity of the crude extracts and isolated pure compounds of these plants. The crude dichloromethane extracts of the plants showed activity against the chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (D 10). The crude plants extracts exhibited activity with IC_{50} values raging from 2.14 µg/ml to 32.5 µg/ml. The pentacyclic triterpenoids (PTCs) isolated and characterized (through IR, NMR, MS spectral data) from the leaves and stem bark of *M. caffra* and *M. obtusifolia* include taraxerol (I), Sawamilletin (II), and ursolic acid (III). Ursolic acid was found to have anti-plasmodial activity (IC_{50}, 6.8 µg/ml). The results validate the use of these plants in folk medicine.

PE37 Bioactivities of Some Herbal Medicines from Zimbabwe

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The development of new drugs is a priority in pharmaceutics in order to ensure human well-being which is being threatened by new diseases, the development of resistance to the existing drugs, and the existence of diseases that are currently incurable. Plant extracts used by traditional healers have not been satisfactorily explored as a source of new drugs. This project aims to assess the bioactivity of some herbs used in Zimbabwe folk medicines, with a view to enhance their contribution to national and international health issues. Forty-five species from 25 plant families, sampled from different provinces in Zimbabwe with the assistance of traditional healers, were identified at the Harare Botanical Gardens and cleared for export by the plant quarantine section of the Zimbabwe Forestry Commission. The parts of the plants used by the traditional healers to treat various diseases were collected for screening for bioactivity. The samples (25 g) were sequentially extracted with dichloromethane, and methanol. Each extract was screened for acetyl cholinesterase inhibition, and antioxidant (DPPH), and antimalarial activity. Seventy-five extracts (83.3%) were active in at least one of the bioassays. The activities were distributed as follows: six DCM extracts (6.7%) and 13 methanol extracts (14.4%) had antioxidant activities (21.1%), while 17 DCM extracts (18.9%) and three methanol extracts (3.3%) exhibited acetyl cholinesterase inhibition (22.2%). Thirteen DCM extracts (14.4%) and four methanol extracts (4.4%) had an IC_{50} of less than 6 µg/mL (18.8%). Only three species (3.3%) reported antioxidant and acetyl cholinesterase inhibition, and antimalarial activity. The results show that some of the herbs have the potential to contribute significantly to the development of new drugs or to new sources of drugs for malaria, Alzheimer's disease, and age-related problems. Work is in progress to identify and isolate pure bioactive compounds from the extracts.

PE38 Effect of different extraction techniques on structural components and complement fixation activity of polysaccharide fractions from *Terminalia macroptera* leaf

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The tree *Terminalia macroptera* is widespread in West Africa. In traditional medicine root, stem bark and leaves have been utilized frequently in the treatment of various diseases. The leaves are used in gastritis, colic and hypertension, against fever, lepra and tuberculosis. Recently, it was confirmed that polysaccharides of certain structures modulate the immune system and intensify its defense mechanism. Three techniques for extraction of leaf polysaccharides were employed: 50 °C and 100 °C water after Soxhlet extraction (SE) with organic solvents; accelerated solvent extraction (ASE) (both organic solvents and water) and boiling water extraction (BWE). Purified polysaccharide fractions with high complement fixating activity were obtained from crude extracts by ion chromatography and gel filtration. The complement fixation ability of polysaccharide fractions were determined to have ICH_{50} values varying between 8.8 and 86.0 µg/mL, and the molecular weights of these fractions varied between 19.8 kDa and 220.3 kDa. The polysaccharide fractions from ASE extraction showed higher complement fixation activity and molecular weights. The monosaccharide compositions and partial structure were also compared. Details in the structures differ, and the difference of the complement fixation ability may relate to the difference of structural properties and will be discussed. Keywords: *Terminalia macroptera*; Soxhlet extraction; Accelerated solvent extraction; Boiling water extraction; Polysaccharide; Complement fixating activity.

PE39 In vitro antihyperlipidemic activity of triterpenes from stem Bark of *Protorhus longifolia* (Benrh) Engl.

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Obesity is a common disorder of carbohydrate and fat metabolism. In an effort to discover new more effective drugs against obesity and its comorbidities, we investigated the *in vitro* antihyperlipidemic activity of two triterpenes (3β-hydroxylanosta-9,24-dien-21-oic acid and methyl-3β-hydroxylanosta-9,24-dienoate isolated from stem bark of *Protorhus longifolia* and characterized through NMR, LC-MS, IR. The inhibitory activity of the triterpenes was evaluated on selected lipid (pancreatic lipase and cholesterol esterase) and carbohydrate (disaccharidases, α-glucosidase) digestive enzymes. The inhibitory activity of the compounds on hormone-sensitive lipase and the ability to bind bile acids were also evaluated. Furthermore, the effect of the compounds on cellular (muscle cells, C2C12 and fat cells, 3T3-L1) glucose uptake was investigated. The triterpenes effectively inhibited the activities of pancreatic lipase, cholesterol esterase, and hormone-sensitive lipase with IC50 values ranging from 26.6 to 430 µg/ml, and showed to varying degree inhibition of maltase and other disaccharidases. The compounds showed moderate bile acid binding ability and at 50 µg/ml, both compounds also mimicked insulin character by effectively stimulating glu-

cose uptake in both the C2C12 and 3T3-L1 cells. It is apparent that the compounds possess hypolipidemic properties.

F. Ethnopharmacology of Amazonian medicine

PF2 Acetylcholinesterase inhibitors from *Tetrapterys mucronata*

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In our search for acetylcholinesterase (AChE) inhibitors, the investigation of *Tetrapterys mucronata* Cav. (Malpighiaceae), a plant used in Brazil for its psychotropic properties, was pursued. The methanolic extract of the bark of *T. mucronata* presented an interesting AChE inhibition in a TLC bioautographic assay. In order to isolate the active compounds, bioassay-guided isolation was undertaken using HPLC-microfractionation in 96 well plates and agar-overlay bioautography to localize the active compounds. The analytical HPLC-PAD conditions were geometrically transferred to a preparative medium pressure chromatography (MPLC-UV) by chromatographic calculations for the efficient isolation of the active compounds at the milligram scale in one step. Using this approach, 22 compounds were isolated, six of them being new natural products, such as 5-hydroxy-2-(2,3-dihydroxy-1-(1 H-indol-3-yl)propyl)-1 H-indole-3-carboxamide and 3-(2-(dimethylamino)ethyl)-2-(2,3-dihydroxy-1-(1 H-indol-3-yl)propyl)-1 H-indol-5-ol. The structure of the isolated compounds was elucidated by classical spectroscopic methods including UV, 2D NMR and HR-MS and chemical derivatisation. Some of the isolated compounds presented a strong AChE inhibition on the bioautographic assay; however those compounds showed weak activity when it was measured in solution.

PF3 Discovery of Plumericin as a novel potent NF-κB inhibitor from *Himatanthus sucuuba*

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The transcription factor nuclear factor kappa B (NF-κB) plays a crucial role in the regulation of the inflammatory response and contributes to the development of various diseases (1). Thus, its inhibition is considered a promising approach to combat inflammation (2). In this study, we applied a bioactivity-guided approach to identify NF-κB inhibitors from the stem bark of *Himatanthus sucuuba*, an Amazonian plant traditionally used to treat inflammation-related disorders. We identified the spirolactone iridoid plumericin as a potent NF-κB inhibitor. Plumericin inhibits NF-κB activation in TNF-α stimulated HEK293 cells stably transfected with a NF-kB-driven luciferase reporter gene (293/NF-κB-luc cells), suppresses TNF-α-induced surface expression of adhesion molecules ICAM-1, VCAM-1 and E-selectin in endothelial cells, and reduces neutrophil recruitment in thioglycollate-induced peritonitis in mice. The effect of plumericin on the NF-κB signalling reveals to be a direct inhibition of the upstream kinase IKK-β. Consequently, it abolishes TNF-α-induced IκB phosphorylation and subsequent degradation. These findings might contribute to the development of promising anti-inflammatory leads and provide scientific evidence for the traditional use of *Himatanthus sucuuba* against inflammatory diseases. **References**: [1] Baker, R.G., Hayden, M.S., Ghosh, S., 2011, Cell Metabolism, 13(1): 11 – 22; [2] Karin, M., Yamamoto, Y., and Wang, Q.M., 2004, Nature Review Drug Discovery 3: 17 – 26 **Acknowledgements:** This work was supported by the Austrian Federal Ministry for Science and Research and the Austrian Science Fund (Drugs from Nature Targeting Inflammation – DNTI project)

PF4 Immunomodulation induced by the aqueous extract obtained from *Ampelozizyphus amazonicus*

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Ampelozizyphus amazonicus Ducke (Rhamnaceae) (popularly known as "Saracura-mirá") is a medicinal plant from Amazonia (Brazil). An aqueous drink is prepared from its barks and roots which displays a very bitter taste and forms abundant foam when shaken, due to the high content of dammarane-type saponins in the species. The plant is used in the region to treat and prevent malaria, as well as a stimulant and energetic. Previous studies did not show a direct action of this plant on *Plasmodium* blood forms, however, it is effective on controlling infection induced by sporozoite forms. In this study, we investigated whether the aqueous extract (SM) obtained from the stem barks of *A. amazonicus* collected in "quilombola" communities of Oriximiná (Para), Brazil, could control malaria infection through an overall augmentation of the immunological response. Daily oral treatment and dose of SM was based on its traditional use (Peçanha et al. 2013). Oral treatment with SM induces an increase in both IgM and IgG antibody titers in mice immunized with the T-independent type 2 (TI-2) antigen TNP-ficoll. We investigated the effect of SM treatment on the course of B cell response in *P. chabaudi*-infected mice and observed that SM treatment induced an increase in the levels of circulating total IgM and IgG in *Plasmodium*-infected mice when compared to infected untreated animals. There was, however, a decrease in the number of antibody-producing plasma cells (CD138+ cells) in the spleen of *P. chabaudi*-infected SM-treated mice. The data obtained in our study indicates that SM could amplify the response of murine B cells to a TI-2 antigen and could also increase immunoglobulin production during malaria infection and regulate the emergence of antibody secreting cells. Future studies on the role of saponins isolated from SM on both B cell response and resistance to malaria infection will be performed. **Reference**: [1] Peçanha LMT et al., BioMed Research International, Vol 2013, Article ID 451679, 11 pages.

PF5 Anti-inflammatory effects of methyl cinnamate, the major constituent of the essential oil of *Ocimum micranthum*, on the gastrointestinal tract of rats submitted to acetic acid-induced colitis

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The essential oil of *Ocimum micranthum* Willd. has analgesic properties in nociception of inflammatory origin and exerts antispasmodic actions on rat airways after inflammatory stimulus (Planta Med. 2012;78:681). Its effects involve methyl cinnamate (MC), a compound potentially interesting for studies involving conjunctly smooth muscle cells, nociception, and inflammation. Thus, this study was designed to observe whether MC impairs the deleterious effects caused in acetic acid-induced colitis. Wistar rats of the colitis group (CG) were instilled with acetic acid (5%) by rectal route, while control group (S) received saline. Animals received (p.o.) saline or MC (50 mg/kg) daily for 3 days after instillation procedure. Positive control was prednisolone (P; 1 mg/kg, i.p.). At the 3rd day, the rats were sacrificed in order to obtain blood samples and colon strips that were disposed in bath chamber to construct concentration-effect curves to KCl (1 – 140 mM). Rats of CG showed intense inflammatory process at visual inspection, which was confirmed by the increased levels of leukocytes in blood samples ($5.6 \pm 0.5 \times 10^3$ cells/μl in S [n = 10]; $8.6 \pm 0.7 \times 10^3$ cells/μl in CG [n = 9]; $p < 0.05$). MC or P significantly decreased the leukocyte levels in CG to $6.4 \pm 0.4 \times 10^3$ cells/μl [n = 9] and $5.4 \pm 0.6 \times 10^3$ cells/μl [n = 6] ($p < 0.05$), respectively. The levels of IL-1β, which were 737.3 ± 81.4 pg/ml (n = 5) in CG, were significantly ($p < 0.05$) decreased by MC and P to 511.0 ± 75.7 pg/mL (n = 5) and 317.4 ± 23.9 pg/ml (n = 6), respectively (S group = 434.1 ± 22.9 pg/ml; n = 7). Maximal contraction induced by KCl

was 0.05 ± 0.01 g/mg of tissue (n = 5) in CG, value significantly lower than 0.21 ± 0.03 g/mg of tissue (n = 5) in S. MC and P reverted partially the detrimental effects on KCl-induced contractions in CG (Emax = 0.14 ± 0.03 g/mg of tissue (n = 5) and 0.11 ± 0.01 g/mg of tissue (n = 6), respectively). Thus, MC has protective effects against the deleterious actions of acetic acid instilled on gastrointestinal tract of rats.

PF6 Myorelaxant effects of methyl cinnamate, the major constituent of the essential oil of *Ocimum micranthum*, on smooth muscle of the gastrointestinal tract of rats

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Methyl cinnamate (MC) is a flavoring compound naturally found in the essential oil of *Ocimum micranthum* Willd. (EOOM), but its effects on gastrointestinal tract are not known. This study aimed to characterize the pharmacological actions of the EOOM (~40% of MC) and MC on the contractile behavior of strips from rat gastrointestinal smooth muscle disposed in bath chambers. EOOM (0.1 to 500 μg/ml) had antispasmodic effects against contractions induced by carbachol (CCh; 1 μM; IC_{50} of 91.9 μg/ml) or KCl (60 mM; IC_{50} of 46.8 μg/ml) in strips of rat stomach fundus, whereas MC inhibited CCh with IC_{50} of 63.6 μg/ml (392.6 [227.8 – 675.9] μM; [geometric mean [95% C.I.]; n = 5) or KCl with IC_{50} of 34.5 μg/ml (213.0 [96.0 – 472.4] μM; n = 8). In strips of gastric antrum, duodenum or large intestine, the inhibitory actions of MC was not significantly different in comparison to that in fundic strips ($p > 0.05$, ANOVA). The myorelaxant effect of MC on CCh-induced contractions was not changed by pretreatment with L-NAME (300 μM; IC_{50} of 382.9 [340.0 – 431.3] μM; n = 6) or tetraethylammonium (TEA; 3 mM; IC_{50} of 399.5 [149.8 – 1065.5] μM; n = 6). In presence of 0.2 mM sodium orthovanadate, a tyrosine phosphatase inhibitor, the CCh-induced contraction was reduced by MC (1 mM) to 43.8 ± 8.2% of the control (n = 11), value significantly higher than 26.7 ± 6.6% (n = 11) observed with MC alone ($p < 0.05$, Student's t test). In Fluo4-loaded freshly isolated smooth muscle cells from large intestine, MC (600 μM) significantly decreased the cytoplasmic level of Ca^{2+} measured by confocal microscopy ($p < 0.05$, Holm-Sidak). Under Ca^{2+}-free conditions, MC (300 μM) also inhibited the intracellularly mediated transient contractions induced by acetylcholine (3 μM) or caffeine (20 mM). In conclusion, MC appears involved in the relaxant effect of EOOM. The effects of MC recruit a decrease in the intracellular levels of Ca^{2+}, being partially blunted by the inhibition of the protein tyrosine phosphatase.

PF7 Antioxidant activity and phenol content and flavonoids total of the *Hymenaea stigonocarpa* Mart and *Hymenaea courbaril* L

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Hymenaea stigonocarpa Mart. *and Hymenaea courbaril* L. var. stilbocarpa (Hayne), family Leguminosae, are medicinal species commonly found in the Brazil, they are widely used in tea form to treat several diseases such as gastric pain, ulcers, diarrhoea and inflammation. The beneficial effects of these species are correlated with the presence of secondary metabolites. In this context, the aim of the present work was to investigate antioxidant activity and the phenolic compounds and flavonoids in methanolic and hydroethanolic extracts from leaf, rind, seeds and pulp of *H. courbaril* Mart. and *H. stigonocarpa* L. fruits. All extracts were obtained by maceration and concentrated under reduced pressure in rotary evaporator. The method used to evaluate the antioxidant activity was the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and the antioxidant activity of the extracts was calculated as the EC50 concentration. The total phenolic content of the extract was determined by the Folin-Ciocalteu test. Quantification was carried out on the basis of the standard curve of commercial gallic acid, and the concentration of total phenolic compounds was expressed as mg of gallic acid equivalents (GAE)/g of dry extract. The flavonoids content was determined by the ultraviolet-visible spectrometry method and calculated from the standard curve of rutin and the results were expressed as mg of rutin equivalents (RE)/g of dry extract. All determinations were performed in triplicate. Among the various extracts evaluated, the hydroethanolic extract of the seed of *H. courbaril L.* presented the maximum contents of total phenolics and total flavonoids (464,34 mg GAE/g of dry extract and 442,25 mg RE/g of dry extract, respectively). This extract also promoted high DPPH scavenging activity (EC 50 = 149,43 (μg/mL). According to the results obtained, extracts with higher antioxidant capacity also has higher total phenolic and flavonoids.

PF8 Plants used for diabetes in the transition zone of Platinum and Amazon Hydrographic Basins, southwest portion of Mato Grosso, Brazil

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Diabetes is a disease of increasing scope. It increasingly affects elderly and almost elderly. People use medicinal plants based on knowledge derived from multiple sources, and thus define the species to treat diseases. The correct use of plants helps take care of health. Aim of this study is to present plants used for diabetes in the transition range Basins Platinum (P) and Amazon (A). The study is part of Project PLAMED/ Group FLOBIO/CNPq research/FAPEMAT/UNEMAT. Was investigated in 2005 in three counties (I. Pontes-Lacerda, II. Vale São Domingos, III. Jauru) located in the transition zone (T = PA) between Platinum (P) and Amazon (A) basins, southwest portion of Mato Grosso, Brazil. The informants were persons of recognized domain in the subject and indicated by the local community. The study provides 11 indications pointing to 9 plant species used for the control of diabetes. Of these, 7 species were identified by informants for most populous municipality T (I). The species identified are the following three groups: G1) Açafrão [*Curcuma longa* L.], Cana-do-brejo [*Costus spiralis* (Jacq.) Roscoe] Maracujá [*Passiflora edulis* Sims.], Alcachofra [*Cynara scolymus* L.] Marmeleira [*Alibertia edulis* (A. Rich.) L. Rich.]; G2) Jucá [*Caesalpinia ferrea* Mart.], Pata-de-vaca [*Bauhinia* sp], Urucum [*Bixa orellana* L.]; G3) Carqueja [*Baccharis* sp]. Additional studies indicate that the plant G3 is also used in adjacent municipalities to T on both sides (P and A); G2 species are used also in municipalities adjacent platinum (P); while plants G1 are indicated specifically in the region of this study (T = PA) compared to the surroundings (P, A). Of the plants identified, species with more scientific studies associated with diabetes are *C. longa* and *C. scolymus* and, with fewer studies, *A. edulis* and *C. spiralis*. We conclude that the studied region (T) is more influenced by the choices and habits of the people of P than by the people of the region A

PF9 Medicinal plants used to treat snakebites by people in the neighborhood watersheds Platinum and Amazon, Brazil

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Illness caused by snakebites, in Brazil, by some authors, are included in the list of "neglected diseases". These accidents occur most frequently in the tropics. Treatments and preventive measures are known, but not available in poorer areas. Therefore there is a high rate of deaths and sequelae. The use of alternative therapies in the treatment of health includes plants and is much more common as are most marginalized community. A higher proportion of snakebites occur in rural areas. In general, in the rural settlements that are less developed, there is no stock of antivenom. The long distances and barriers to rapid deployment to find appropriate care contribute to the worsening of the patient, leading to many cases of death. This set of situations induces populations at high risk to recover and generate knowledge in the community to address their demands. They define local resources as well, such as

the use of plants to treat the injured, which are believed to have efficacy. Anti-ophidian plants well regarded and used by people who live in the neighboring region of Platinum and Amazon basins are presented. In 2005, 21 municipalities in southwestern Mato Grosso (Brazil) were visited to collect data interviewing 63 informants considered the most knowledgeable in the subject and indicated by the local community. We wanted to know which herbs were the most important and for what purpose they served. In five counties by 5 informants, in all, six plant species were found that are used to treat snakebites, including: "Quiabo" (*Abelmoschus esculentus* (L.) Moench.), "Coentro" (*Coriandrum sativum* L.), "Guiné-Santo" (*Petiveria alliacea* L.), "Negramina" (*Siparuna guianensis* Aubl.), "Batata-doce" (*Ipomoea batatas* L.), "Beijo-branco" (*Impatiens balsamina* L.). However, in April 2013, there were few scientific studies available online, associated with these plants to treat snakebites. This encourages us to deepen studies on this focus, to scientifically support this use.

PF10 Cytotoxic activity against tumour cell lines and anti-inflammatory effects of compounds isolated from *Xylopia aromatica*

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Plants of the Annonaceae family have been used in traditional medicine for the treatment of a variety of ailments including diarrhoea, fever, pain and inflammation. Bioassay-guided separation of a *Xylopia aromatica* leaf extract led to the isolation of two labdane diterpenoids whose structures were identified by 1 H, 13C NMR spectroscopy as 8(17),12E,14-labdatrien-18-oic acid (**1**) and 15,16-dihydroxy-8(17),13(E)-labdadien-19-oic acid (**2**), These diterpenoids were tested for their cytotoxic effect against the human HT29 and MDA-MB-231, and mouse 4T1 cancer cell lines. Using a 48 h sulphorhodamine B assay, compounds **1** and **2** exhibited relatively low GI_{50} (growth inhibition) values of 9.4 and 7.5 µg/mL respectively, compared to 7.1 µg/mL for cisplatin. The anti-inflammatory effects of the compounds were tested in terms of their ability to inhibit nitric oxide (NO) production by LPS-activated RAW 264.7 macrophages over 24 h. These compounds also inhibited NO production at lower concentrations (IC_{50} values: 1 – 13.5 µg/mL, 2 – 12.3 ug/mL, aminoguanidine [control] – 10.5 µg/mL), but without showing an important cytotoxic effect (IC_{50} > 200 ug/mL) using the (3-(4,5-dimetiltiazol-2yl) -5- (3-carboximetoxifenil) -2(4-sulfofenil) -2 H-tetrazolium) (MTS) cytotoxicity assay. From the inactive fractions six known compounds were isolated: quercitrin, quercetin 3,3' dirhamnose, liriodenine, lysicamine, xylopianin and annomontacin.

PF11 Anti-inflammatory and anti-tumour effects of two species of Cat's Claw (*Uncaria*)

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South America has two predominant species of Cat's Claw (Uña de Gato), *Uncaria tomentosa* and *U. guianensis*, which are used in traditional medicine to treat inflammation. In this study, a commercial preparation of *U. tomentosa* (Samento) and a hydroethanolic preparation of *U. guianensis* were evaluated to determine their potential anti-inflammatory and anti-tumour activities. An ethyl acetate-soluble fraction, containing flavonoids and alkaloids, was further fractionated (Sephadex LH20 and HPLC). Two preparations showed promising activity, the compound SamA1 (*U. tomentosa*) and the subfraction UgAIV (*U. guianensis*). UgAIV inhibited nitric oxide (NO) production by LPS-activated RAW 264.7 macrophages *in vitro* (85% at 30 mg/ml) and in mice 1 h after challenge with LPS (79% at 5 mg/Kg). UgAIV also inhibited the TNF-a, IL-6 and prostaglandin E_2 responses in the same *in vitro* and *in vivo* models, as well as inhibiting NF-kB activation (70% at 30 mg/ml). Cox-2 expression was inhibited by UgAIV (36%) and iNOS by SamA1 (50%), both at 30 mg/ml. In RAW 264.7 macrophages, IkB degradation was 100% inhibited with SamA1 (30 mg/ml), with total inhibition of NF-kB translocation. The preparations reduced 4T1 mammary tumour growth at day 15 by 62% (SamA1) and 49% (UgAIV), reaching a maximum of 67% (SamA1) on day 20 and 91% (UgAIV) on day 33 post-inoculation. Tumour-bearing mice treated with the preparations showed serum NO, IL-6 and TNF-a at less than 50% of the controls. UgAIV decreased the number of tumour-infiltrating T lymphocytes, macrophages and neutrophils by more than 50%. UgAIV and SamA1 decreased the number of cell positive for COX-2, iNOS, IL-6, TNF-a and P65 in the infiltrate by more than 45%. The anti-inflammatory effect of UgAIV and SamA1 is possibly due to inhibition of NF-kB and the anti-tumour effect may be due to a reduction in pro-tumoural inflammatory processes in the tumour microenvironment. Identification of the active compound(s) is under way.

PF12 Anti-inflammatory effects of (+)-catechin isolated from the bark of *Byrsonima crassifolia*

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Inflammation is a defence mechanism which is generally self-limiting, but there is an on-going search for anti-inflammatory drugs to treat chronic inflammatory conditions where the inflammatory response is inappropriate and harmful. Ever since the medicinal use of plants containing salicylates were first reported in Egyptian times, the search has continued to identify secondary metabolites in plants with immunomodulatory, anti-oxidant or anti-inflammatory activities. Initial screening of a number of plants collected in Venezuela led to the identification of *Byrsonima crassifolia* (Malpighiaceae) as an inhibitor of inflammatory mediators in its crude bark extract form. This plant has been traditionally as an antiemetic, diuretic, febrifuge, and to treat diarrhoea, gastritis and ulcers. Bioactivity-guided fractionation of the crude extract using solvents of different polarities, followed by HPLC, led to the isolation of a flavonoid which showed the greatest activity in several bioassays, nitric oxide (NO – 50% inhibition at 37 µg/ml) and prostaglandin E2 (40% inhibition at 100 µg/ml) production by LPS-stimulated Raw 264.7 cells, serum NO in LPS-challenged mice (56% inhibition at 40 mg/Kg) and paw oedema in the mouse carrageenan model (80% inhibition at 40 mg/Kg). The flavonoid did not significantly inhibit either TNF or IL-6 production *in vitro* or *in vivo*. MS and NMR analysis of the flavonoid identified it as (+)-catechin, for which anti-oxidant activities have been reported. However, as far we are aware, this is the first report of its activity against the inflammatory processes described here.

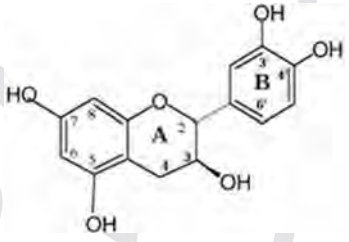

Fig. 1

PF13 Screening of Venezuelan plants for anti-inflammatory activity. Results from an *in vitro* nitric oxide assay

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Due to its geographical location in the north of South America, Venezuela is considered to be one of the richest and varied countries in terms of plant diversity. Estimates of the total flora of Venezuela near 30,000 species, of which more than 1,500 are used for medicinal purposes by indigenous and local communities. However, only a relatively small proportion of these have been evaluated in terms of their anti-inflammatory activity. Our botanical database (MedPlant) now contains over 4,000 collections representing over 2,000 species. Most of the plants were collected in three states of Venezuela, Amazonas, Bolívar and Cojedes, from 1999 to the present. In this study, we evaluated 165 extracts from 92 species for inhibition of nitric oxide production (Griess assay) by the LPS- stimulated monocyte/macrophage cell line RAW 264.6, as a possible indicator of anti-inflammatory activity. The most promising extracts (50% inhibition of NO at < 40 µg/ml, without cytotoxic effect) were from *Tapirira guianensis, Jacaranda copaia, Oxycaryum cubense, Croton cuneatus, Cochlosperm vitifolium, Datura inoxia, Hamelia patens,*

Ipomoea carnea, Machaerium madeirense, Euterpe precatoria and *Costus scaber*. Some of these have reported anti-inflammatory activity (*Croton cuneatus*, *Cochlosperm vitifolium, Hamelia* patens), while others, such as *Datura inoxia, Tapirira guianensis* and *Oxycaryum cubense* have not been reported to show such activity until now. A selection of these plants is now under study using bioassay-guided fractionation.

G. Computational methods in natural products chemistry

PG1 DFT molecular modelling of novel cadinane sesquiterpenes isolated from *Nectandra amazonum*

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Nectandra is a genus of plants especially abundant in neotropical regions including 114 species. It has been demonstrated that some Nectandra plants exhibit antitumoral, antioxidant, antiinflammatory, febrifuge, and hypotensive activities attributed to the presence of alkaloids and neolignans in crude extracts of Nectandra, suggesting good potential as chemotherapeutics. However, sesquiterpene-related compounds are also highly recurrent in some Nectandra, whose structures possess mainly germacrene, eudesmane, and cadinane moieties. Thus, as part of our research on Colombian Lauraceae plants, a carefully phytochemical exploration was carried out on *n*-hexane-soluble sub-extract from *N. amazonum* leaves-derived EtOH extract. Repeated chromatography of the above-mentioned sub-extract yielded two new cadinane-related sesquiterpenes (**1 – 2**), along with two known sesquiterpenes (**3 – 6**), whose chemical structures were established by analyses of spectroscopic data (1D and 2D NMR, MS) in comparison with spectroscopic data in the literature. The interesting structural features of the new compounds **1 – 2** will be presented on the basis of spectroscopic analyses. A DFT (density functional theory) molecular modeling study at the B3LYP level was separately performed on **1 – 2** starting from a NOESY-resulted configuration in order to support the assignments, due to the conformation-dependent variability of sesquiterpenes. Thus, according to the resulting Boltzmann population analyses for optimized structures of **1** and **2**, the lowest stable conformers exhibited a chair-conformed B-ring and supported the NOESY correlations. The novel sesquiterpenes were identified as rel-(4S,6S)-cadina-1(10),7(11)-diene (**1**) and rel-(1R, 4S, 6S, 10S)-cadina-7(11)-en-10-ol (**2**). The unusual unsaturation profile of compound **1** suggests further studies which are required to define chemomarkers in order to clarify the biosynthetic role of such sesquiterpenes in Nectandra.

PG2 New sesterterpenoids from *Salvia mirzayanii* – stereochemical characterization by computational electronic circular dichroism

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Sesterterpenes are rare in nature and have been reported mostly from marine sponges and algae. Among terrestrial plants, *Salvia* species are a good source for these compounds. They exhibit diverse biological properties, such as anti-inflammatory, cytotoxic, anti-biofilm, antimicrobial, and anticancer activities. In a project directed at novel bioactive metabolites from endemic Iranian Lamiaceae, we studied *Salvia mirzayanii.* We isolated five new sesterterpenoids, whose structures were secured by means of extensive NMR (1D and 2D) and MS spectroscopy. Structure elucidation revealed that compounds **1-3** only differ in their configurations at C-13 and C-14. Assignment of relative and absolute configurations was challenging due to free rotation around the C-13/C-14, but could be achieved by comparison of experimental and simulated ECD spectra of all possible stereoisomers by using time dependent density function theory TDDFT/CAM-B3LYP/6 – 31G** in the MeOH using the "self-consistent reaction field" method (SCRF) with the conductor-like polarizable calculation model (CPCM). [1 – 2] Absolute configuration of **4** and **5** were established in a similar manner.

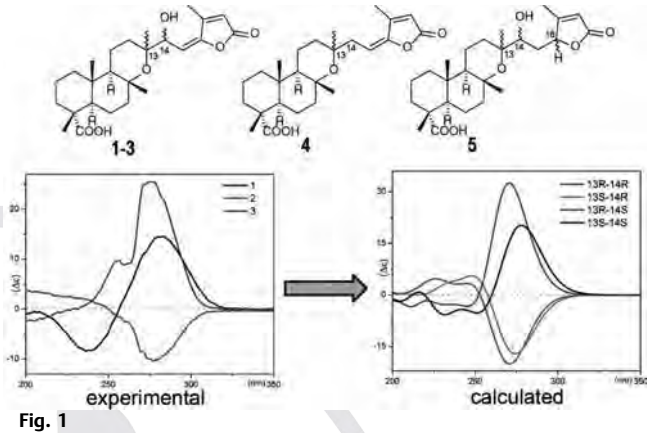

Fig. 1

References: [1] Bringmann, G.; Gulder, T. A. M.; Reichert, M.; Gulder, T., *Chirality* **2008**, *20*, 628 – 642. [2] Moradi-Afrapoli, F.; Ebrahimi, S.N.; Smiesko, M.; Raith, M.; Zimmermann, S.; Nadjafi, F.; Brun, R.; and Hamburger, M.; Phytochemistry, **2013**, 85, 143 – 152

PG3 New insights into the anti-influenza activity of licorice constituents

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Neuraminidase (NA), a key enzyme in viral replication and spread, is the first-line drug target to combat influenza. Based on a shape-focused virtual screening approach, the roots of *Glycyrrhiza glabra* L. (licorice) were identified as plant material with an accumulation of molecules that show 3D similarities to previously discovered influenza NA inhibitors [1]. Moreover, licorice is the most frequently cited herb in TCM for treating respiratory tract infections [2]. Phytochemical investigations revealed twelve constituents identified as (E)-1-[2,4-dihydroxy-3-(3-methyl-2-butenyl)phenyl]-3-(8-hydroxy-2,2-dimethyl-2 H-1-benzopyran-6-yl)-2-propen-1-one (**1**), 3,4-dihydro-8,8-di-methyl-2 H,8 H-benzo[1,2-b:3,4-b']dipyran-3-ol (**2**), biochanin B (**3**), glabrol (**4**), glabrone (**5**), hispaglabridin B (**6**), licoflavone B (**7**), licorice-glycoside B (**8**) & E (**9**), liquiritigenin (**10**), liquiritin (**11**), and prunin (**12**). A distinct inhibition of the cytopathic effect in MDCK cells was observed for compounds **3** and **5** (IC_{50}s 40.3; 37.4 μM). On the target level, chemiluminescence (CL)-based NA inhibition assays were performed using the NA of different influenza virus strains including A/342/09 (H1N1), an oseltamivir-resistant virus isolate. Strikingly, 11 compounds showed IC_{50}s in the low micromolar to even nanomolar range. For most constituents 2- to 10-fold higher concentrations were necessary to inhibit the NA of the oseltamivir-resistant virus. In addition, the NA of *Clostridium perfringens* was shown to be susceptible in CL- as well as fluorescence-based assays. In this work, we report novel insights into the anti-influenza potential of licorice constituents which includes also a critical discussion of possible assay interference problems [3].

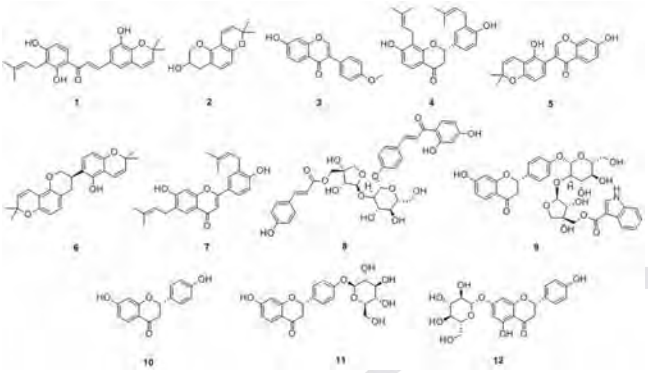

Fig. 1

References: [1] Grienke 2010, J. Med. Chem. 53, 778 [2] Ge 2010, Nat. Prod. Rep. 27, 1758 [3] Chamni 2013, Expert Opin. Ther. Patents 23, 409
This work is supported by the Austrian Science Fund (FWF: P24587 & P23051) and the European Social Fund (ESF & TMWAT Project 2011 FGR 0137).

PG4 *In silico* docking researches of some human proteins with anthranoids

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Anthranoids have diverse biological effects in the cell through interactions with proteins like receptors, enzymes or transporter proteins [1]. Anthranoids and their effect mechanisms have been a major focus of research activities recently. Still their molecular targets and mechanism of their therapeutic activities are poorly understood. In this study, angiotensin converting enzyme (ACE) [2], casein kinase II α (CKIIα) [3], human myosin light chain kinase member 4 (MYLK4) [4] were selected as targets for main anthranoids from hundreds of scanned proteins as being potential molecular targets of anthranoids by using an *in silico* docking approach [5] and then computationally determined their relative binding energies to anthranoids isolated from *Rumex patientia* (Polygonaceae) and *Rhamnus frangula* (Rhamnaceae). The main anthranoids were docked and docking results were compared. According to the docking results for CKIIα and human MYLK4 emodin and rhein anthraquinone aglycones showed the lowest binding energy respectively wheras glucofrangulin B showed the lowest binding energy at the interaction with ACE. This work aims to determine important anthranoids with wide range of activity including several important receptors or enzymes. These molecules offering a broad therapeutic window may become novel therapeutic agents of future potential drugs. **References:** [1] Srinivas G, Babykutty S, Sathiadevan PP, Srinivas P (2007) Medicinal Research Reviews 27: 591 – 608 [2] Hyun SK, Lee H, Kang SS, Chung HY, Choi JS (2009) Phytother Research 23: 178 – 184 [3] Litchfield DW (2003) Biochem J 369: 1 – 15 [4] Greenman C, Stephens P, Smith R et al (2007) Nature 446: 153 – 158 [5] Trott O, Olson AJ (2010) J Comput Chem 31: 455 – 461

PG5 Theoretical study of structural α-and β-cubebin isolated from *Aristolochia esperanzae* Kuntze

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Rheumatoid arthritis (RA) causes severe physical disabilities. Ethnopharmacological studies indicate use of *Aristolochia esperanzae* Kuntze in treating RA. Phytochemical analysis of the stems of *A. esperanzae* provide a mixture of β-cubebin (**1**) and α-cubebin (**2**). This paper presents calculation results in different methods for structural analysis of these substances and of research of preferred positions of interaction between the molecule (**2**) and solvents. The calculations were performed using the software package of Gaussian 03W[1]. Chemical shift calculations were performed using different methods and structures in gaseous state without intermolecular interactions. The data obtained with optimized geometries in different methods have been correlated with experimental 13C-NMR shift data that are in literature[2]. The best correlation coefficients (R^2, Table 1) indicate most appropriate method to study of these substances.

Tab. 1: Correlation coefficients (R^2) obtained for **1** and **2**

Compound/Method	Level of calculation			
	HF	BLYP	B3LYP	PBE
1	0,98449	0,98676	0,99739	0,99119
2	0,98437	0,98521	0,99667	0,98665

The best level of calculation (B3LYP/6 – 31G*), indicated by better correlations, was used to determine the geometry of isolated phytochemicals – (8*R*,8'*R*,9*S*)-cubebin (**1**) and (8*R*,8'*R*,9*R*)-cubebin (**2**). The effect of the solvent was investigated by the explicit addition of one molecule of solvent acetone, dimethylsulfoxide and pyridine in different positions of interaction with (**2**) at the B3LYP/6 – 31G* level. The systems of (**2**) with solvent showed high correlation coefficients. The configurations with the best correlations indicate the positions of preferential interaction between this molecule and solvent (Figure **1**).

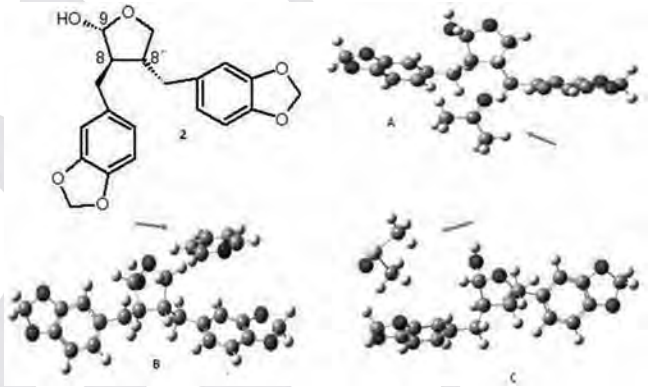

Fig. 1: Structure of (8*R*, 8'*R*, 9*R*)-cubebin (**2**) and preferred positions of interaction between (**2**) and solvents acetone (A), pyridine (B) and dimethylsulfoxide (C).

Acknowledgements: CNPq, UFMG, and IFSULDEMINAS **References**: [1] *Gaussian 03, Revision C.01.* [2] Pascoli, I. C.; Nascimento, I. R.; Lopes, L. M. X. *Phytochemistry* **2006**, *67*, 735. Christiansson, A.; Bertilsson, J.; Svensson, B.

PG6 Medicinal plants used for diabetes in municipalities of the Amazon Basin, the southwestern portion of Mato Grosso, Brazil

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The use of medicinal plants is an alternative to complementary therapy aimed at health care. Diabetes is a metabolic syndrome that affects more and more people, and causes irreversible damage in many victims. Local people use plants based on knowledge from multiple sources, and thus define the list of plants to treat each disease. We present plant species used to control diabetes in municipalities of the Brazilian Amazon Basin. The study is part of Project PLAMED/FLOBIO/CNPq/FAPEMAT/UNEMAT and includes four counties "(Vila Bela da Santíssima Trindade, Conquista do Oeste, Nova Lacerda, Comodoro)" located in the Amazon Basin (A), the southwestern portion of Mato Grosso, Brazil. Data collection was performed in 2005. Informants, visited and interviewed, were persons of recognized reference in the subject and indicated by the local community. This study discloses the use of nine plant species for control of diabetes. Of these nine, five species ["Estomalina" (*Vernonia condensata* Baker), "Fedegoso"(*Senna occidentalis* (L.) Link), "Hortelã do campo" (*Hyptis suaveolens* Poit.), "Serralha" (*Sonchus oleraceus* L.), "Carovinha"(-*Jacaranda caroba* (Vell.) A.DC.)] were indicated in the municipality of "Vila Bela da Santíssima Trindade" (1818), the oldest, which is further south, two species ["Babosa" (*Aloe vera* (L.) Burm. F.), "Infalivina" (*Artemisia vulgaris* L.)] in the municipality "Comodoro" (1986) further north, while the younger municipalities (1990 s), located between the two already mentioned, were appointed the "Azeitona-preta" (*Syzygium cumini* L.) in "Nova Lacerda" (1995) and the "Carqueja" (*Baccharis* spp.) in "Nova Lacerda" and "Conquista do Oeste" (1999) counties. There are more scientific studies available online in Apr. 2013, associated with

diabetes, for *A. vera* and *S. cumini* and less for *J. caroba* and *V. condensata*. There is a diverse choice of herbal antidiabetics in this region, suggesting further studies on their efficacy and safety.

PG7 Fast virtual screening of sesquiterpene lactones from Asteraceae with potential antileishmanial activity

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Leishmanioses is a human tropical parasitic disease that causes approximately 50,000 death cases annually. Secondary metabolites play an important role to propose new active lead structures, and studies highlights antiprotozoal activities of sesquiterpene lactones (SLs)[1]. In view of this, we performed a virtual screening (VS) in an in-house databank of 1328 SLs of Asteraceae, corresponding to 2323 botanical occurrences (B.O. – number of times that a compound appears in different species) using fragment descriptors and Randon Forest (RF). We select from CHEMBL database a diversity set of 269 compounds, which were screened against *L. donovani* amastigotes (MHOM/ET/67/L82) to generate a RF model and were classified using -logIC_{50} (mol/L)= pIC_{50} values, being active (> 5) and inactive (< 4.5) and, between 4.5 and 5 were excluded to reduce border effect between both classes. DRAGON v. 6.0 generated descriptors, which with constant and near constant values, standard deviation < 10^{-4}, and pair correlation ≥ 0.90 were excluded. The 148 remaining descriptors and class variable were exported to Knime 2.7.2 that was used to perform all analysis process described hereinafter. Data were divided in train and test set and only 7 variables were selected by backward feature elimination method. RFs were generated using WEKA nodes. **Table 1** summarizes match rates of RF of 269 compounds of CHEMBL database. RF selected 476 SLs (658 B.O.) with potential activity which are present mainly in Senecioneae and Eupatorieae tribes. The VS, that is part of the activities of ResNetNPND (http://www.uni-muenster.de/ResNetNPND/), is rapid and can be applied to larger natural products databases.

Tab. 1: Summary of match rates.

	Train			Validation[a]		Test		
	Samples	Match	%Match	Match	%Match	Samples	Match	%Match
Active	97	95	97.8	78	80.4	24	22	91.7
Inactive	118	116	98.3	101	85.6	30	23	76.7
Overall	215	211	98.1	179	83.3	54	45	83.3

a – cross-validation (10 stratified groups)

References: [1] Schmidt J, *et al. Curr. Top. Med.*, 2012, 19, 2128 – 2175.

H. Hyphenated analytical techniques and target fishing

PH1 High-end analytical technology for the valorization of apolar natural products out of bio-waste: an integrated workflow

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Organic-biological waste produced by the food industry often contains natural components of high value including health promoting phytonutrients that are barely utilized from these resources today. This is among others due to the fact that this bio-waste is not well characterized on the molecular level. An essential first step in a potential valorization process or optimal use of resources is knowing their composition. Therefore a generic screening platform was developed on an Ultra High Performance Liquid Chromatograph-Photodiode Array Detector-accurate mass-Mass Spectrometer (UHPLC-PDA-am-MS) for the measurement of apolar plant metabolites in biological matrices. For am-MS detection Atmospheric Pressure Chemical Ionisation (APCI) was combined with orbitrap technology (Exactive – Thermo Scientific). Because of the generic character of the sample preparation procedure and the use of screening detectors, compound detection is virtually only limited to its solubility and ionisation efficiency. Therefore a wide range of interesting natural compounds (e.g. carotenoids, phytosterols, fat soluble vitamins, polyacetylenes, etc.) can be identified simultaneously without the use of analytical standards (e.g. 115 identified apolar plant metabolites in chili pepper). Interesting identified unknowns were extracted from samples and purified by Supercritical Fluid Chromatography (SFC) and, subsequently, their identities were confirmed by Nuclear Magnetic Resonance (NMR) analysis. The purified compounds could then be applied as analytical standards for quantification purposes, thereby aiding the assessment of the viability of potential end products (cosmeceuticals, pharmaceuticals, food supplements) developed from bio-waste or even aiding the optimization of biomass culturing conditions for maximum phytonutrient production. Future perspectives are the sustainable and economically viable extraction of valuable plant metabolites out of food processing waste for product development.

PH2 Improving method reliability through selective removal of glycerolipid and chlorophyll interferences

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Exact quantitative determination of carotenoids and other secondary plant metabolites is essential in the estimation of potential valorisation pathways. Erroneous quantification can cause major differences in the end result of an economical calculation and can make the difference between potential profit or loss. A generic Accelerated Solvent Extraction (ASE) procedure was optimized for the complete extraction of carotenoids from organic biological material. As the extraction procedure is generic, other apolar compounds like glycerides and chlorophylls are also extracted, which can induce ionization suppression of co-eluting carotenoids in mass spectrometric detection or can disturb ultra violet detection. Extra purification steps are therefore necessary to minimize matrix influence of triglycerides and chlorophyll derivatives on detection. Saponification is generally used to hydrolyse glycerolipids and chlorophylls. However, in nature carotenoids are regularly esterified with fatty acids and saponification also hydrolyses these esterified carotenoids. The natural composition of carotenoids present in the sample is therefore lost with implications on future valorisation steps. Lipase enzymes were evaluated to selectively remove glycerolipids like di- and triglycerides from the sample matrix, leaving esterified carotenoids untouched. Lipases are enzymes that are naturally present in animals for the digestion of glycerolipids. The optimized lipase clean-up method resulted in hydrolysis of more than 96% of triglycerides present in the samples. For the selective removal of chlorophylls, preparative open bed column chromatography, selective removal of chlorophylls using dioxane and a new selective 'Chlorofiltr™' (UCT) adsorbent were investigated. The optimized extraction method was coupled to the clean-up steps, resulting in an effective generic sample preparation flowchart for the qualitative and quantitative screening of apolar metabolites in organic biological samples.

PH3 Identification and quantification of bioactive polyacetylenes in supermarket products and food processing waste

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Food plants of the *Apiaceae* family such as carrots, celery and parsley contain C 17-polyacetylenes that have shown to be highly toxic towards fungi and bacteria and to preferentially kill human colorectal cancer cells and inhibit tumour growth. Therefore these compounds could potentially be used as food preservatives, natural fungicides or even new anticancer agents. However, no analytical standards are commercially available for these compounds which hinders their identification and quantification in potential resources. Therefore a generic screening method for the detection of apolar plant metabolites on an Ultra High Performance Liquid Chromatograph-Photodiode Array Detector-accurate mass-Mass Spectrometer (UHPLC-PDA-am-MS) was used for the tentative identification of polyacetylenes in vegetable samples (carrot, celery, parsnip). For am-MS detection Atmospheric Pressure Chemical Ionisation (APCI) was combined with orbitrap technology (Exactive – Thermo Scientific). Several polyacetylenes were detected. Two polyacetylenes, falcarinol and falcarindiol, were ubiquitous. Therefore falcarinol and falcarindiol were selected for in-house standard production. They were purified out of a parsnip extract with a semi-preparative Supercritical Fluid Chromatograph-PDA-MS (purity ≥ 97%). Subsequently, their identities were confirmed by Nuclear Magnetic Resonance (NMR) analysis. The purified compounds were applied as analytical standards for quantification of these polyacetylenes in supermarket products and real food processing waste (e.g. steam peels, etc.) with the UHPLC-PDA-am-MS method. The polyacetylenes were found to be widely present in supermarket products and bio-waste. Quantification of these compounds allows bio-waste selection for product development and assessment of the viability of potential end products. Future perspectives are the sustainable and economically viable extraction of polyacetylenes out of bio-waste for product development (e.g. natural fungicides).

PH4 Identification of new pro-apoptotic inhibitors of HSP70

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Oridonin is a very promising anti-cancer *ent*-kaurane diterpene.[1 – 4] Recently we have demonstrated that the pro-apoptotic activity of oridonin depends on its ability inhibit the activity of HSP70.[5] It is a member of a ubiquitously expressed family of molecular chaperones involved in de novo protein folding, subcellular trafficking, proteasome-mediated degradation, and autophagy. At the initial stages of tumorigenesis, HSP70 has been shown to protect the cells undergoing transformation from oncogenic stress induced by overexpression of oncogenes. HSP70 overexpression has been routinely associated with poor prognosis in multiple forms of cancer and is thought to provide a survival advantage to cancer cells interacting with multiple cellular pathways.[6] On the basis of this data we started a systematic study by surface plasmon resonance on diterpenes structurally related to oridonin in order to find out new HSP70 inhibitors. Our investigations demonstrated that 15-ketoatractyligenin methylester (SR2017), the semi-synthetic derivative of the *nor*-diterpene atractyligenin, binds with high affinity the chaperone. This interaction was also confirmed by chemical proteomics experiments performed on Jurkat cell protein extracts. We also demonstrated the ability of SR2017 to inhibit the growth of tumor-derived cell lines with higher potency than oridonin. Moreover, we found that this compound causes mainly cell cycle impairment, inducing cells to accumulate in S/G_2-M, also leading to cell death by apoptosis. These results are in agreement with a possible inhibitory effect of SR2017 on HSP70 and suggest this compound as a new promising anti-cancer agent. **References:** [1] Sun H.D. et al. *Nat Prod Rep.* **2006**, *23*, 673 – 698. [2] Cai D.T. et al. *Mol Cell Biochem.* **2013**, in press [3] Wang S. et al. *Am J Chin Med.* **2013**, *41*, 177 – 196. [4] Sun K.W. et al. J Gastroenterol. **2012** *18*, 7166 – 7174. [5] Dal Piaz F. et al. *J Proteom.* **2013**, *82*, 14 – 26. [6] Evans C.G. et al. *J Med Chem.* **2010**, *53*, 4585 – 4602.

PH5 Application of multivariate statistical analysis for the discovery of compounds with *c-Myb*-inhibitory activity in extracts of *Juglans* species

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The identification of bioactive compounds within an extract has always been a capital aim of phytochemical research. Up to now, bioactivity guided fractionation has been the most popular method to achieve this aim, a method characterized by high expenses regarding time and resources. However, the development of fast analytical methods, having taken place during the last decades, finally allows an alternative and reformative approach to the active constituents of an extract. In this project, partial least squares regression (PLS) has been applied to different extracts of *Juglans* species in order to discover the constituents responsible for their *c-Myb* inhibitory activity. *c-myb*, a proto-oncogene and transcription factor, is supposed to be of relevance for the hematopoietic system. The deregulation of *c-myb* is connected to certain kinds of cancer i.e. leukemia, breast and colon cancer. *c-Myb* inhibitors are considered interesting as new leads against these diseases[1]. In the course of the present study, extracts of *Juglans* sp. have been proven to possess *c-Myb* inhibitory activity in a cellular assay, based on doxycyclin-induced *c-Myb* activity coupled with GFP-mediated fluorescence. Samples were taken from different plant materials of diverse *Juglans* species at variable times in the vegetation period and different extraction methods were applied to obtain a variety of crude extracts. UHPLC-/ESI-qTOF-MS was employed to generate analytical fingerprints of these extracts. IC_{50} values of the *c-Myb* inhibition were determined in the assay mentioned above. Using multivariate statistics software (Unscrambler), PLS models were constructed correlating the activity with analytical data. These models allowed localizing compounds in the LC/MS chromatograms which are most likely responsible for the *c-Myb* inhibitory activity. Targeted isolation of these compounds is in progress. **Reference:** [1] C. Schomburg, W. Schuehly, F.B. Da Costa, K.-H. Klempnauer, T.J. Schmidt. Eur. J. Med. Chem. 63, 313 – 320 (2013)

PH6 Metabolomics study of hydroxytyrosol's administration in a metabolic syndrome rat model

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Metabolic syndrome is a clustering of interrelated risk factors for cardiovascular disease and diabetes. The Mediterranean diet has been proposed as an important dietary pattern to confer cardioprotection by attenuating risk factors of metabolic syndrome. Hydroxytyrosol (HT) is a single phenol present in large amount in olive byproducts and in lower amounts in olive oil and in olive fruits, which are basic constituents of the Mediterranean diet. In the context of identifying the features of HT that are responsible for its effects on the metabolic syndrome, an experimental protocol has been setup encompassing isolated HT administration in a diet induced model of metabolic syndrome in young Wistar rats. Rats were randomly divided into four groups as cornstarch (CS), cornstarch + HT (CSHT), high-carbohydrate high-fat (HF) and high-carbohydrate high-fat + HT (HFHT) (n = 6/group). HT (20 mg/kg/d oral gavage, water vehicle) was administered for 8 weeks on the basal diet (CS

or HF). In order to gain insight on the metabolic effects of HT administration on the total biochemical profile (metabolome), an untargeted approach (metabolomics) has been developed and attempted on the rat plasma and urine samples from the metabolic syndrome model. The new biomarkers discovered from multivariate analysis have been identified by comparison with public domain metabolome libraries but also by confirmation using HRMS/MS, highlighting the differences in the biochemistry of HFHT animals versus controls as well as versus CSHT. Fatty acid metabolism was found to be down regulated in HFHT rats while glycerol, which releases from triacylglycerols, was the main up regulated metabolite in HFHT rats and correlates to fatty acid transportation catabolism. HT supplementation for 8 weeks reduced visceral obesity and was associated with improved left ventricular structure and function, reduced blood pressure, improved glucose disposal and reduced hepatic steatosis.

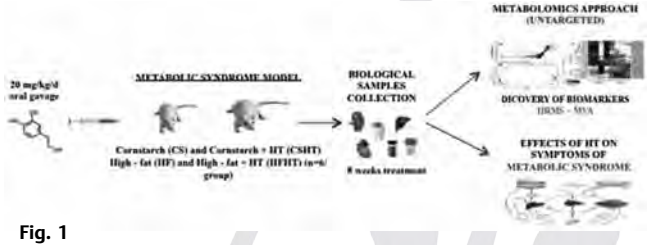

Fig. 1

PH7

Preparative metabolite isolation of *Tropaeolum majus* L. by high-speed countercurrent chromatography based on *off-line* continuous ESI-MS/MS injection

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Nasturtium or Indian cress (*Tropaeolum majus* L., Tropaeolaceae) native to Latin America is traditionally used to treat cardiovascular disorders, respiratory diseases, and bacterial urinary tract infections. Bioactive secondary metabolites are glucosinolates, with its principal marker glucotropaeolin, polyphenols including derivatives of quercetin, and kaempferol, as well as phenolic acids [1 – 3]. In our study, preparative *high-speed countercurrent chromatography* (HSCCC) was used to fractionate compound classes from a MeOH macerate of *T. majus* leaves for further biological evaluations. The complex metabolite profile of a 900 mg crude extract injection to HSCCC was monitored by *off-line* ESI-MS/MS with automatic continuous injections in the order of the recovered HSCCC fractions. The resulting reconstituted HSCCC/ESI-MS profile visualized target-compound areas, enabled a most accurate fractionation, and also supplying MS/MS data of 5 selected precursor ions. Selective ESI-MS ion traces monitored complex co-elution effects, as well as chromatographic sections with pure compounds which were recovered for NMR [4]. Cinnamic acid quinic acid esters (CQAs), and flavonoid glycosides such as quercetin-3-*O*-(6''-*O*-malonyl)-glucoside, or quercetin-3-*O*-glucoside were recovered on preparative scale.

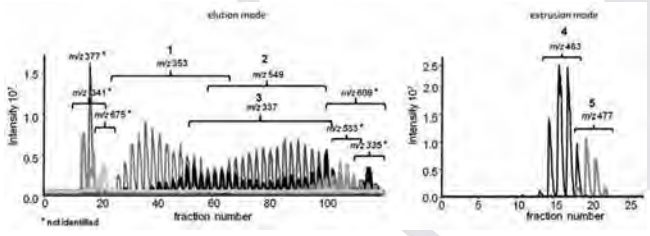

Fig. 1: Reconstituted *HSCCC-ESI-MS/MS* profile of *T. majus* (neg. mode, *m/z* 100 – 1500) with selected ion traces $[M-H]^-$ at *m/z* 353: 5-chlorogenic acid (**1**), *m/z* 549: quercetin-3-*O*-(6''-*O*-malonyl)-glucoside (**2**), *m/z* 337: 5-coumaroyl quinic acid (**3**) in the *elution mode*. The *extrusion mode* yielded *m/z* 463: quercetin-3-*O*-glucoside (**4**), and *m/z* 477: kaempferol-3-*O*-glucoside **(5)**.

References: [1] Franz, *Z. Phytother.* **1996**, 17, 255 – 262. [2] Gasparotto et al. *J. Ethnopharmacol.* **2009**, 122, 517 – 522. [3] Hegnauer, R., *Chemotaxonomie der Pflanzen (Bd. VI)* Birkhäuser Verlag, Basel **1973**. [4] Jerz et al., In: Recent Advances in the Analysis of Food and Flavors, pp 145 – 165. ACS Symp. Series 1098; **2012**.

PH8

Preparative mass-spectrometry profiling of genotoxic pyrrolizidine alkaloids in *Jacobaea vulgaris* (syn. *Senecio jacobaea)* by *spiral-coil countercurrent chromatography* and ESI-MS/MS off-line continuous injection

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Pyrrolizidine alkaloids (PAs) are genotoxic natural products. Almost 350 PAs are known with respective *N*-oxides. Legal regulations of PAs in food, and feed are discussed. There is a lack of available reference substances for targeted quantification [1]. PA-contamination was reported for honey, and bee products [2,3]. PA-contamination of retail ready-to-use lettuce was demonstrated. Morphological similarities of rocket salad (*Diplotaxis tenuifolia,* syn.: *rucola),* and the PA-plant *Senecio vulgaris* resulted in unintentional co-harvesting. In our study, PA-extract of *J. vulgaris* flower heads (Asteraceae) was fractionated by prep. *spiral-coil countercurrent chromatography* (*spCCC*, 5.7 L coil). The system *n*-hexane – EtOAc – MeOH – H_2O (3:7:5:5) was used to separate 5 g of crude alkaloid extract. Obtained fractions were sequentially *off*-line injected to ESI-MS/MS. This *target-guided* isolation procedure [4] made use of selected ion traces of PAs. Elution of major (**2**, **5**, **7**), and minor (**1**, **3**, **4**, **6**) PAs were monitored (altern. mode MS^2, *m/z* 100 – 1500). The *spCCC elution* resulted in the fractionation of PAs, and one non-PA (**8**) in the *extrusion*-mode (**8** not presented) [5]. Almost pure compounds were obtained for PAs **1**, **2**, **5**, **7**, and the non-PA **8** could be used as reference materials. Minor PAs were enriched and will be further purified with other separation techniques.

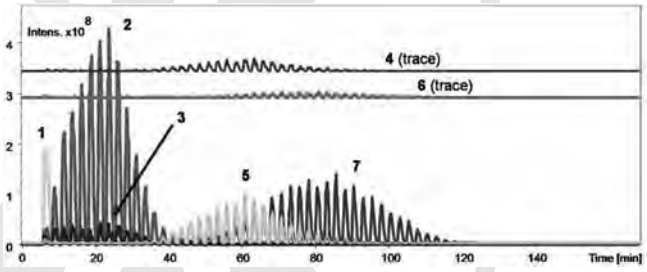

Fig. 1: PAs from the *spHSCCC* run monitored by cont. injections to ESI-MS^2 with selected ion traces $[M+H]^+$ *m/z* 398 (**1**) not id., *m/z* 350 erucifoline (**2**), *m/z* 352 jacobine (**3**), *m/z* 564 (**4**) not id., *m/z* 334 senecionphylline (**5**), *m/z* 392 acetyl-erucifoline (**6**), *m/z* 336 senecionine (**7**).

References: [1] BfR, Nr. 038/2011, *http://www.bfr.bund.de/cm/343/analytik-und-toxizitaet-von-pyrrolizidinalkaloiden.pdf* [2] Kempf et al., *Mol. Nutr. Food Res.* 2010, 54, 158. [3] Kempf et al., *Food Addit. Contam., Part A,* 2011, 28, 325. [4] Jerz et al., In: *ACS Symp. Series 1098*; 2012. [5] Fragoso-Serrano et al., *J. Nat. Prod*. 2012, 75, 890.

PH9

RP-HPLC-DAD/MS/MS-based metabolic profiling of glycogen phosphorylase inhibitory active fractions of *Nauclea latifolia* stem bark

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Nauclea latifolia Smith (Rubiaceae) are commonly prescribed in African traditional medicine as a remedy for a number of diseases including diabetes mellitus.[1] Although a series of experimental animal studies have established its antidabetic activity little is known about the possi-

ble mechanism of its action.[2, 3] This prompted us to investigate the *in vitro* inhibitory action of the methanolic extract of *N. latifolia* stem bark and fractions therefrom on human liver glycogen phosphorylase (HLGP). *N. latifolia* stem bark methanolic extract and the chloroform and ethyl acetate fractions significantly inhibited HLGP at a concentration of 5 mg/ml with 85%, 69% and 98%, respectively. On the other hand, i*n vitro* screening with 2,2-diphenyl-1-picrylhydrazyl (DPPH) at concentration of 12.5 µg/ml revealed a prominent radical scavenging activity (RSA) of the chloroform fraction (85%) and relatively lower RSA of the methanolic (68%) and ethyl acetate fraction (77%) which seems not to be closely correlated with anti-diabetic activity. RP-HPLC-DAD coupled with tandem mass spectrometry performed on the ethyl acetate fraction which was associated with the most significant HLGP inhibitory activity resulted in the identification of chlorogenic acid, its glucoside, caffiec acid dimer, noengenin and noengenin dimmer, apigenin7-O-pentouronic acid, quercetin, luteolin 7-methoxy, isorhamnetin and isorhamnetin 3-methoxy **References:** [1] Gidado, A.; Ameh DA.; Atawodi, SE. and Ibrahim. S. Hypoglycaemic activity of *Nauclea latifolia* Sm. (Rubiaceae) in experimental animals. *Afr. J. Trad. CAM,* 2008, 5 (2), 201 – 208. [2] Gidado, A. and Atawodi, S E. Effect of *Nauclea latifolia* leaves aqueous extract on blood glucose levels of normal and alloxan induced diabetic rat. *African Journal of Biotechnology,* 2005, 4(1), 91 – 93. [3] Iwueke, AV. and Nwodo, FO. Antidiabetic effect of *Sarcocephalus latifolus* aqueous root extract in experimental rat model. *Animal Research International.* 2007, 4(2), 698 – 701.

Tab. 1: *in vivo:* inhibition of carrageenan-induced inflammation

Treatment (dose) *(i.p)*	Net oedema Volume (ml)		% Inhibition
	Control	Treated	
Extract (1 g/kg)	0.7 ± 0.04	0.27 ± 02	61.6
Hydrocortisone (20 g/kg)	0.7 ± 0.04	0.35 ± 0.03	50.1
Diclofenac (20 mg/kg)	0.7 ± 0.04	0.49 ± 0.06*	30

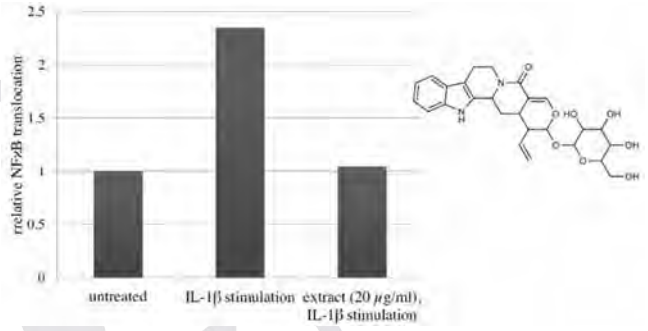

Fig. 1: *in vitro:* inhibition of NFκB translocation Strictosamide as causative agent

PH10 Anti-inflammatory properties of *Nauclea latifolia* extracts and isolation of strictosamide by a high-content screening approach

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Crude extracts of *Nauclea latifolia* (Rubiaceae), commonly known as pin cushion tree, are employed in traditional or folk medicine and are used in the treatment of several diseases, such as malaria, gastrointestinal tract disorders, and hypertension. We were able to demonstrate that treatment with the root bark ethanolic extract of *N. latifolia* inhibited the formation of edema in a rat inflammation model. Carragenan-induced inflammation was inhibited by 62% after 1 h treatment (i.p.) with the crude extract at 1 g/kg. In addition, administration (i.v.) of the ethanolic extract decreased the blood pressure in rats that were treated with arachidonic acid. Following bioactivity-guided screening we aimed at characterizing single plant metabolites that are responsible for the activity observed with crude extracts. In a high-content screening approach using the stable CHO/NFκBp65-GFP cell line we found that the crude extract of *N. latifolia* (4 h treatment, 20 µg/ml) completely inhibited the IL-1β induced translocation of the transcription factor NFκB from cytoplasm to nuclei. Further, we found this effect to be induced by certain HPLC fractions of the chloroform extract of *N. latifolia*. We were then able to identify strictosamide as the active ingredient and could validate our results in reporter gene assays. HEK293T/17 cells were transiently transfected with a reporter construct that contains five copies of an NFκB response element that drives transcription of a luciferase transporter gene. At a concentration of 50 µg/ml strictosamide inhibited the IL-1β induced transcription by approximately 70%. Previously, it was reported that strictosamide may account for the folk use of plant extracts on hypertension. However, the compounds mechanism-of-action remains to be elucidated. We assume that strictosamide, at least to a certain extent, accounts for the anti-inflammatory properties of the crude *N. latifolia* extracts observed in rat models.

PH11 UHPLC-ESI(+)-HRMS-based metabolic profiling as a dereplication tool for the detection and identification of *Acronychia*-type acetophenones in crude extracts

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Acronychia-type acetophenones constitute a characteristic group of constituents of *Acronychia* species. *Acronychia* species have been traditionally used in folk medicine for their anti-inflammatory and antipyretic effects to treat asthma, ulcers and rheumatism. Previous studies have demonstrated the biological importance of *Acronychia*-type acetophenones as significant cytotoxic principals[1]. However, their analysis and identification via chromatographic and spectroscopic/spectrometric techniques have not been reported up to now. Therefore, in the present study, a sensitive and specific method using an UHPLC-ESI(±)-HRMS platform was developed for the separation, detection and structural characterization of *Acronychia*-type acetophenones in crude extracts. Specifically, a UHPLC apparatus hyphenated to a hybrid LTQ-Orbitrap MS equipped with an ESI ionization probe, in positive and negative mode was incorporated. Chromatographic (Rt) and spectrometric features such as suggested ECs and RDBeq. values in both full scan and MS^n level as well as data dependent acquisitions were employed. Fragment ions generated in MS^2 and MS^3 level at *m/z* 341.1719, 285.1095 and 229.0469 were found characteristic for *Acronychia*-type acetophenones and could be utilized for the identification thereof. Moreover, diagnostic ion ratios were determined for the identification of acetophenones isomers and different substitution patterns. In order to evaluate the validity of the generated method for dereplication purposes, diverse species and organs of *Achronychia* genus were collected from Malaysia and Vietnam and extracted. Applying the developed method, known acetophenones were successfully detected together with unknown ones. Additionally, supervised and unsupervised data analysis methods were used to facilitate the identification procedure and support the findings. **Reference**: [1] Kouloura et al, Cytotoxic Prenylated Acetophenone Dimers from *Acronychia pedunculata*, J. Nat. Prod, 2012, 75 (7):1270 – 6

PH12 Profiling of natural products by HPLC combined with DAD and accurate in-source ESI-TOF-MS spectra followed by TLC/HPLC/DAD/ESI-TOF-MS bioactivity screening

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Natural products play important and increasing role in pharmaceutical market. In many cases, in comparison with synthetic drugs side affects are less prominent. They are also often a source of new lead compounds, used solely, or for semi-synthesis. Due to its sensitivity and efficiency, HPLC hyphenated with DAD and MS detection systems is widely used in searching for potentially new natural compounds present in plant materials. Nowadays, accurate MS analysis plays special role in rapid qualitative research of potentially novel plant metabolites. Because of instrumental developments with double mass calibrations systems used in modern time-of-flight mass spectrometers (TOF-MS), it is possible to determine exact molecular mass within mass errors even less than 2 ppm. Therefore, errors in molecular formula confirmations have been minimized. In our research we developed HPLC combined with DAD and accurate ESI-TOF-MS for rapid and efficient dereplication of known metabolites and discovery of potentially new natural compounds. Plant materials were extracted using modern pressurized liquid extraction (PLE), followed by solid-phase extraction (SPE) and then HPLC/DAD/ESI-TOF-MS with accurate in-source MS-fragmentation. In this way, variety of alkaloids (eg. Amaryllidaceae alkaloids), coumarins, chromones, flavonoids and phenolic acids were discriminated in plant samples from Amaryllidaceae, Umbelliferae and Compositae families. Several potentially novel compounds could be determined. Fragmentation pathways were also established, and enabled for example determination of different groups among Amaryllidaceae alkaloids. Moreover, TLC with bioauthography utilizing novel instrumental set up: TLC/HPLC/DAD/ESI-TOF-MS was used for screening of acetyl- and butyrylcholinesterasase inhibitors among investigated plant extracts.

PH13 Sex specific aspects in the treatment of inflammation induced gene expression of cytokines by the multi-herbal drug STW 5 in colonic preparations

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Sex and gender aspects are important in understanding differences between men and women in their risk and experience of gastrointestinal disorders. Relative to men, women are diagnosed more frequently with irritable bowel syndrome (IBS). It has been discussed that genetic modifications in IL 10 expression could be responsible for the sex differences. The aim of the present study therefore was to determine in gene expression of anti-inflammatory and pro-inflammatory cytokines and to evaluate the effect of the multi-herbal drug STW 5 (Iberogast®) on the gene expression in untreated and inflamed colonic preparations from male and female rats. Comparisons were made with preparations from rats of the same parents. The rats were held in a special room of the animal house to exclude environmental factors. The colonic preparations were pretreated with 2,4,6-trinitrobenzenesulfonic acid (TNBS 10 mM). TNBS induces severe colonic inflammation involving lamina propria mononuclear cells. The gene expression (qRT-PCR) of the proinflammatory cytokines TNF-α and IL 6 as well as the anti-inflammatory cytokine IL 10 was more pronounced in untreated preparations of female rats compared to preparations from male rats. After preincubation with TNBS the gene expression of the cytokines was drastically increased (TNF-α 3.9, IL 6 4.2, IL 10 3.0 fold) in male preparations whereas the increase in female preparation was moderate and ranged from 0.8 (IL 10) to 1.4 (IL 6) fold. STW 5 reduced the TNF-α gene expression and STW 6, a constituent of STW 5, increased the IL 10 gene expression. The detailed sex specific analysis indicated that these effects were only present in preparations of male rats which had low levels of TNF-α and IL 10 genes. Our results clearly indicated sex differences in the cytokine regulation of colonic preparations from male and female animals while an inflammation is induced by TNBS and these could be important for the likewise different action of STW 5 in this model.

I. Natural product chemistry

PI1 Five triterpenoidal saponins from the butanol fraction of *Balanites aegyptiaca* Del

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The butanol fraction of the 70% ethanolic extract of *Balanites aegyptiaca* fruit was fractionated using vacuum liquid chromatography (VLC RP-18 column). A gradient elution was performed using a mixture of methanol/water, yielding 5 sub-fractions monitored by analytical high performance liquid chromatography (HPLC). The sub-fraction eluted with 75% methanol was subjected to a preparative HPLC where five triterpenoidal saponins were isolated for the first time from *Balanites aegyptiaca* fruit. The isolated compounds were identified as: **compound 1**, 26-O-β-D-glucopyranosyl-(25R)-furost-5-ene-3,22,26-triol 3-O-[α-L-rhamnopyranosyl-(1→3)- β-D-glucopyranosyl-(1→2)- α-L-rhamnopyranosyl-(1→4)] β-D-glucopyranoside; **compound 2**, 26-O-β-D-glucopyranosyl-(25R)-furost-5-ene-3,22,26-triol 3-O-[β-D-glucopyranosyl-(1→2)- α-L-rhamnopyranosyl-(1→4)] β-D-glucopyranoside; **compound 3**, 26-O-β-D-glucopyranosyl-(25R)-furost-5,20-diene-3,22,26-triol 3-O-[α-L-rhamnopyranosyl-(1→3)- β-D-glucopyranosyl-(1→2)- α-L-rhamnopyranosyl-(1→4)] β-D-glucopyranoside; **compound 4**, 26-O-β-D-glucopyranosyl-(25S)-furost-5,20-diene-3,22,26-triol 3-O-[β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)] β-D-glucopyranoside; **compound 5**, 26-O-β-D-glucopyranosyl-(25R)-furost-5,20-diene-3,22,26-triol 3-O-[β-D-glucopyranosyl-(1→2)- α-L-rhamnopyranosyl-(1→4)] β-D-glucopyranoside, by spectroscopical analysis [MS,1D and 2D NMR (HSQC, HMBC, DQF-COSY, HSQC-TOCSY)].

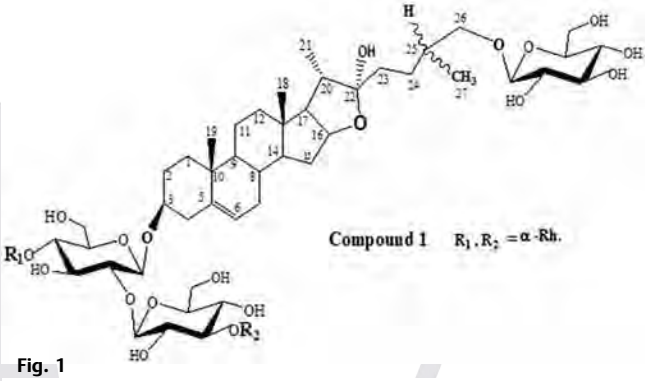

Fig. 1

Acknowledgement: This work was carried out in frame of an FP7 project, Marie Curie Actions, PIRSES-GA-2008 – 230816. Project title is "Natural antidiabetic and anti-hypertensive drugs (NAAN)" and Prof. Rudolf Bauer is the project coordinator

PI2 Chemical constituents from *Solanum glabratum* Dunal var. *sepicula*

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In the course of screening program of Saudi plants for potential cytotoxic activity, the methanolic extract of *Solanum glabratum* Dunal var. *sepicula* and its different fractions were tested against prostate cancer (PC3) and colon cancer (HT-29) cell lines using the MTT assay. In the present study, three spirostan saponin and a flavonoid glycoside were isolated from the active n-butanol fraction through a bio-guided fractionation approach. Two new saponin glycosides were identified as 23-β-D-glucopyranosyl spirost-5-en-3, 23 diol 3-*O*-[α-L-rhamnopyranosyl-

(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside and spirost-5-en-3-ol 3-*O*-[α-L-rhamnopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside. In addition, two known compounds were also isolated and identified as spirost-5-en-3, 23 diol 3-*O*-[α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside and isorhamnetin-3-O-α-L-rhamnopyranosyl (1 – 6) β-D-glucopyranoside. The structures of the isolated compounds were elucidated based on their MS, one dimensional and extensive two dimensional NMR spectral data. Among the isolated metabolites, compound spirost-5-en-3-ol 3-*O*-[α-L-rhamnopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside showed the highest cytotoxic activity against both PC3 and HT29 cell lines with IC_{50} values of 14.8 and 19.5 mg/ml, respectively.

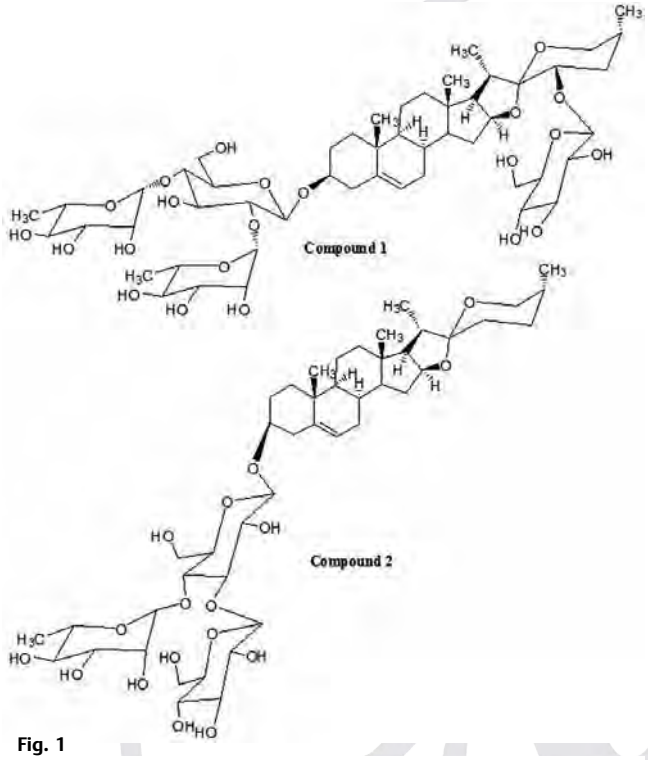

Fig. 1

PI3 Chemical and biological studies on *Tamarix nilotica* growing in Egypt

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Tamarix nilotica (Ehrenb.) Bunge (Tamaricaceae) has been used since Pharaonic times to expel fever, relieve headache, draw out inflammation and as an aphrodisiac agent. *In vitro* antioxidant activity of extracts prepared from the leaves and flowers of *T. nilotica* was tested. Three *in vitro* antioxidant assays were used: 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, superoxide anion scavenging activity and iron chelating activity. Both extracts showed significant *in vitro* antioxidant activity. The hydro-alcoholic extract (80%) of *T. nilotica* flowers was evaluated for *in vivo* hepatoprotective and antioxidant activities. Hepatoprotective activity was assessed using carbon tetrachloride-induced hepatic injury in rats by monitoring biochemical parameters such as serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and alkaline phosphatase. Antioxidant activity was evaluated in alloxan-induced diabetic rats. The hydro-alcoholic extract of *T. nilotica* flowers (100 mg/kg body weight) ameliorated the adverse effects of carbon tetrachloride and returned the altered levels of biochemical markers near to the normal levels. Phytochemical investigation of the leaves of *T. nilotica* has led to isolation of methyl ferulate 3-*O*-sulphate for the first time from natural sources. Coniferyl alcohol 4-*O*-sulphate, kaempferol 4′-methyl ether, tamarixetin and quercetin 3-*O*-β-D-glucupyranuronide were isolated from the *n*-butanol soluble fraction of the extract. The pentacyclic triterpenoid, 3α-(3″,4″-dihydroxy-*trans*-cinnamoyloxy)-D-friedoolean-14-en-28-oic acid was isolated from the *n*-hexane soluble fraction of the extract. The structures of these compounds were determined on the basis of spectroscopic analyses. The isolated triterpene showed the highest DPPH radical scavenging activity with IC_{50} 21.2 μM.

Fig. 1

PI4 Screening of selected Nigerian ethnomedicines for inhibition of β- haematin formation

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Malaria chemotherapy as recommended by the World Health Organisation (WHO) is based on combination of drugs such as the Artemisinin-based Combination Therapy (ACT) (WHO, 2008). This regimen though widely used and effective has drawback of incompatibility in pharmacokinetics (Fidock *et al*, 2004), pre-existent parasite resistance and high cost (Onwujekwe, 2004). This makes the discovery of new compounds (Kurosawa *et al*, 2000) that inhibit formation of β- haematin, the most frequently used drugs in combination with the artemisinins, important. Selected components (25) of recipes used traditionally to treat malaria in South-western Nigeria have been screened for inhibition of β-haematin formation after the modified methods of Ncokazi and Egan (2005) and Vargas *et al* (2011). Active methanol extracts were identified and selected by visual examination (net formation of bright pink colouration) and absorbance measurement on a SoftMax Pro 5.4 plate reader. Most active extracts identified were *Newbouldia laevis* Seem leaf, (Inhibitory value, I_{value} 0.88 ± 0.39), *Rhaphiostylis beninensis*, (Hook. f.) Planch leaf (I_{value} 0.23 ± 0.15). (Chloroquine phosphate, I_{value} 0.72 ± 0.19. The I_{value} measures the inhibition of β-haematin formation; positive values are selected and highest magnitude equals the absorbance of the initial concentration of haematin used in assay). The bioactivity guided fractionation and isolation of selected active extracts using standard chromatographic methods is on-going and will be presented. **References:** [1] Fidock DA et al, (2004). *Nature reviews*, *3*, 509 – 520 [2] Kurosawa Y, *et al* (2000) *Antimalarial agents* and *Chemotherapy 44* (10), 2638 – 2644 [3] Onwujekwe O, *et al* (2004) *Acta Tropica*, 101 – 15 [4] Ncokazi KK and Egan TJ (2000) *Analytical Biochemistry 393*, 303 – 319 [5] Vargas S, *et al* (2011) *J Pharmaceutical and Biomedical Analysis*, *56*, 880 – 886 [6] WHO (2008). World Health Organization, Geneva

PI5 An unusual coumarin derivative from *Polygala boliviensis* A.W. Benn (Polygalaceae)

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Polygalaceae is a family composed by 19 genus and about 1300 species and they are distributed all around the world but it occurs especially in

tropical and subtropical regions. In Brazil there are registered approximately 240 species distributed in 7 genus[1,2]. Polygalaceae main chemical components are saponins, xanthones, alkaloids and volatile oils. Methyl salicylate is considered the main compound present in their essential oils. Besides there are a significant pharmacological and biological activities described for extracts and pure compounds[3]. *Polygala boliviensis* A.W. Benn (syn *P. alfredi* Chodat) is a plant easily found in all South America[4] but to date there are not studies dealing with its chemical composition. So, this work describes the preliminary chemical study of MeOH extract of aerial parts and roots of this species. The extract was partitioned between MeOH/H_2O and hexane, $CHCl_3$ and EtOAc. The extracts obtained were submitted to antioxidant evaluation and toxicity employing brine shrimp test. The $CHCl_3$ extract showed better results. This extract was submitted to silica gel CC and it permitted to isolate a new coumarin. The structure of this new coumarin was determinated by spectral analysis such as mass spectroscopy, IR and NMR (mono and bidimensional). The unusual substituent bearing C-7 and C-8 was identified by the chemical shifts observed in the ^{13}C NMR (including DEPT) and the long range correlations observed in the HMBC. The methyl salicylate present in the aerial parts of this plant was identified and quantified employing HPLC/DAD and external standards. **Acknowledgements:** *We wish to thank CNPq and FAPESB/PRONEM (Brazil) for financial support.* **References:** [1] Marques, M. C. M.; Peixoto, A. L. (2007) *Rodriguésia,* 58: 95 – 146. [2] Paiva, J. A. R. (1998), *Fontqueria*, 50: 346. [3] da Rocha, J. L. C. et al. (2012) *Quimica Nova,* 35: 2263 – 2266. [4] Coelho, V. P. M. et al. (2008*) Acta Botanica Brasílica*, 22: 225 – 239.

PI6 Megastigmanes and phenolic compounds from *Dioclea virgata* (Rich.) Amsh. (Leguminosae)

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Dioclea is a genus which embraces about 50 species and they are distributed in the tropics, especially in forests of Central and South America[1]. The interest in species of *Dioclea* is due the presence of lectins showing anticancer activity[2]. In the folk medicine they are employed in the treatment of different diseases[3,4]. *Dioclea virgata* (Rich.) Amsh is a climber plant popularly known in Brazil as "cipó de anauerá", "cipó pixuma", "feijão bravo", "feijãorana" and "mucuna". To date just *D. grandiflora, D. lasiophylla and D. violacea* present some phytochemical studies which permitted to isolate bioactive compounds[5]. Preliminary studies with the $CHCl_3$ extract of the leaves of *D. virgata* showed antinociceptive activity[6]. The MeOH extract of leaves of *D. virgata* was diluted in H_2O/MeOH (9:1) and partitioned between $CHCl_3$, and successively with AcOEt:H_2O. The $CHCl_3$ extracts was submitted to different chromatographic techniques (CC over sílica gel, flash, Sephadex LH-20) that led to the isolation of (6*S*,7*E*,9*R*)-6,9-dihydroxy-megastigman-4,7-dien-3-one 9-*O*-β-glucopyranoside and (6*S*,7*E*,9*R*)-6,9-dihydroxy-megastigman-4,7-dien-3-one, besides other flavonoids and phenolic acid derivatives[7]. The structures of the isolated compounds were elucidated through data analysis of IR spectra, ^1H and ^{13}C NMR (BB and DEPT) and bidimensional techniques as well (COSY, HMBC and HMQC). **Acknowledgements:** *We wish to thank CNPq and FAPESB/PRONEM (Brazil) for financial support*. **References:** [1] Perez, G., Hernandez, M., Mora, E. (1990), *Phytochemistry*, 29: 1745 – 1749 [2] Pinto, V. P. T. et al. (2010), *Journal of Cancer Research and Experimental Oncology*, 2: 54 – 59 [3] Barreiros, A. L. B. S. et al. (2000), *Phytochemistry*, 55: 805 – 808 [4] Almeida, R. N., et al. (2000), *Pharmaceutical Biology*, 38:394 – 395 [5] Alves, C. Q.; et al. (2009), 32ª RASBQ, Fortaleza-CE [6] Mota, V. G., et al. (2011), *Journal of Biomedicine and Biotechnology*, 1: 1 – 10 [7] Alves, C. Q. et al. (2010), *Planta Medica*, 76:P368

PI7 Phytochemical analysis of biological active methanolic extract of cultivated *Pelargonium graveolens* using countercurrent chromatography conjugated with sephadex column

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Pelargonium graveolens L'Hér. is an important cultivated, aromatic plant with a high worldwide production. It's essential oil has several important applications in aromatotherapy and traditional medicine. Although the essential oil has been extensively studied, there are limited works regarding its polar constituents. Recently a research article showed that both non- and polar extracts exhibit antioxidant and antimicrobial activity and could be considered as an alternative to 'synthetic food preservatives[1]. Countercurrent chromatography (CCC) has become an important tool for the modern phytochemical analysis, while the easy and effective scaling-up capabilities enable its industrial exploitation. In the current study the methanolic extract of *P. graveolens* was investigated. 400 mg of phenolic part obtained from the crude extract using resin Amberline XAD-4 were fractionated by FCPC using EtOAc/ButOH/H_2O (10:5:15, v/v/v) biphasic system and subsequently the selected fractions were submitted to CC using sephadex LH-20. As a result, 3 simple phenolic compounds and 11 flavonoid derivatives were isolated and identified. The structure elucidation of isolated metabolites was confirmed by NMR and HRMS techniques. Additionally, five flavonoid glucosides, present as minor constitutes in the extract, where determined via UHPLC-UV/DAD-ESI-HRMS analysis. It is important to note that the main compounds of the methanolic extract were rutin and quercetin 3-O-(2-O-β-D-xylopyranosyl)-β-D-glucopyranoside. To our knowledge, this is the first complete phytochemical analysis of the polar content of *P. graveolens* and the first separation procedure including CCC employment regarding the genus *Pelargonium*. In conclusion, the polar fraction of rose-scented geranium is a rich source of compounds with high antioxidant capacity and CCC could be efficiently utilized for scaling-up and commercial application. **Reference:** [1] Boukhris M, et al (2012), Phytother. Res., doi: 10.1002/ptr.4853.

PI8 Phenolics compounds produced by *Camarops* sp. an endophytic fungus from *Alibertia macrophylla* (Rubiaceae)

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In our search for bioactive compounds from endophytic fungi from brazilian flora, *Camarops* sp, an endophyte in *Alibertia macrophylla* (Rubiaceae) showed new and bioactive eremophilane sesquiterpenes when cultivated in corn. These observations led us to grow *Camarops* sp. in Czapek (semisynthetic medium for cultivation of fungi and composed by sucrose and inorganic salts) in order to investigate the influence of culture medium in the metabolic production. The fungus was cultivated for 28 days on Czapek and then subsequently extracted with ethyl acetate furnishing the crude extract. The ethyl acetate extract was fractioned by flash chromatography column on reversed-phase C-18 silica, followed by reversed-phase HPLC, to yield nine phenolic compounds namely 4-hydroxyphenyllactic acid (**1**), phenyllactic acid (**2**), 4-hydroxybenzaldehyde (**3**), 4-hydroxybenzoic acid (**4**), 2,4-dihydroxyisobutylbenzoate (**5**), 2,4-dihydroxypentylbenzoate (**6**), 2,4-dihydroxyhexylbenzoate (**7**), 2,4-dihydroxyoctylbenzoate (**8**) 2,4-dihydroxynonylbenzoate (**9**). The compounds **5-8** were identified in mixture by using HREIMS, ^1H, ^{13}C NMR, 2D NMR (HMQC, HMBC, COSY and NOESY). The structure elucidation of **1 – 9** was achieved by 1D and 2D NMR and mass spectroscopic data. Compounds **4-9** have been isolated from *Camarops* sp. for the first time and may be involved in antioxidant activities[1] observed in crude extract produced by *Camarops* sp.. No eremophilane sesquiterpenes were observed in Czapek medium. The isolated phenolic compounds are under biological investigation. **Reference:** [1] Habec, T. R. Prospecção Química e Biológica do Fungo Endofítico *Camarops* sp. Isolado de *Alibertia macrophylla*. MSc. Degree, IQ/UNESP, Araraquara, SP, Brazil, 2012. **Acknowledgements:** FAPESP, CAPES and CNPq

PI9 Microwave-assisted hydrodistillation and extraction of petals from *Rosa damascena* and *Crocus sativus*

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The region of Kozani, in northern Greece, is known for the cultivation of two unique aromatic plants that yield valuable natural products: *Rosa damascena* (Rosaceae) is commercialized for its rose oil and rose water while *Crocus sativus* (Iridaceae) is renowned for its red stigmas, an exclusive aliment (saffron). Several pharmacological properties, including antibacterial, antioxidant, antitussive and anti-diabetic have been attributed to rose petals. Crocus petals have displayed antioxidant and antibacterial activity and were found to reduce blood pressure and be effective in the treatment of mild to moderate depression. The aim of this work was to investigate the phytochemical profile of extracts deriving from the petals of these two plants, obtained by microwave-assisted-hydrodistillation (MAHD) and -extraction (MAE) techniques. Fresh rose petals were distilled with MAHD using various extractions times for the recovery of the rose oil which was analyzed with GC-MS. Citronellol and geraniol were found to be the main components. The aqueous residue that remained inside the clevenger apparatus was fed continuously to macroporous resins (XAD-4) in order to study the remaining phenolic compounds. Dried crocus petals were extracted by applying various extraction times in MAE with ethanol or aqueous ethanol. The optimal extract, in terms of yield, was fed continuously to macroporous resins (XAD-4) for the recovery of the phenolic compounds. The extracts were analyzed, concerning their phenolic constituents with HPLC-DAD and UHPLC-UV. A Folin-Ciocalteau assay was used for the determination of their total phenolic content and their antioxidant potential was evaluated in a DPPH assay. Further chromatographic separation for the purification of the principal bioactive compounds indicated that the rose extract was rich in glycosides of quercetin and kaempferol while the crocus extract contained mostly kaempferol 7-*O*-glucopyanosyl-3-*O*-sophoroside and kaempferol 3-*O*-sophoroside.

PI10 Phytochemical investigation and anti-inflammatory property of ethanol-water extract of the roots of *Anthocleista djalonensis* A. Chev. (Loganiaceae)

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Anthocleista djalonensis A. Chev.(Loganiaceae) is used in traditional medicine for the treatment of various diseases. The plant is known for its antipyretic, stomachic, analgesic and purgative actions. The aqueous ethanol extract of the root is used traditionally for the treatment of inflammatory disorders. Preliminary phytochemical screening of the plant root material gave positive result for tannins, saponins, and carbohydrate. The pulverized root material was extracted with aqueous ethanol (50%) and the crude product was investigated for anti-inflammatory activity using carrageenan induced paw oedema model.[1] The crude aqueous ethanol extract was further partioned into three different fractions (chloroform, ethyl acetate and butanol). Purification of the butanol fraction on column chromatography eluting with chloroform-methanol (95:5) yielded a viscous light brown liquid which was identified as sweroside after spectroscopic analysis (^{1}H and ^{13}C NMR) and comparison with literature data.[2] The anti-inflammatory activity of the isolated sweroside was also carried out. The crude product showed 60.5% inhibition of rat paw edema at the dose of 400 mg/kg, and 37% at 200 mg/kg. The pure compound produced 73.4% inhibition of rat paw edema at 100 mg/kg while indomethacin (positive control) at 10 mg/kg showed 74.5% inhibition under the same experimental condition. The use of the root extract of *A. djalonensis* in the treatment of inflammatory disorders in ethnomedicine is to a reasonable extent validated by this study.

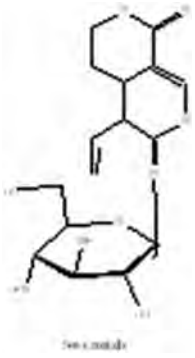

Fig. 1

References: [1] Winter CA, Risley EA, and Nuss GW. Anti-inflammatory and anti-pyretic activities of indomethacin, 1- (p-Chlorobenzyl) -5-methoxy-2-methylindole 3-acetic acid. Journal of Pharmacology and Experimental Therapeutics, 141 (1963), 369 – 376.s [2] Onocha PA, Okorie DA, Connolly JD, Roycroft DS. Monoterpine diol, Iridoid Glucoside and Dibezo-α pyrone from *Anthocleista djalonensis* Phytochemistry, 40 (1995) 1183 – 1189.

PI11 Flavonoids and iridoids from *Asperula lilaciflora* Boiss

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Some members of the genus *Asperula* are consumed as diuretic, for constipation as well as tonic [1]. Flavonoids, anthraquinones, iridoids, phenolic acids as well as phytosterols have been reported from various *Asperula* species [2,3]. In this study, we have examined the constituents of *Asperula lilaciflora*, endemic to Turkey, as contribution to the chemistry of the genus and for future chemotaxonomic consideration. Several chromatographic techniques performed on the MeOH extract of the aerial parts of *A. liliaciflora* led to the isolation of a new flavonol glycoside named as lilacifloroside (quercetin 3-*O*-[6'''-*O*-3,5-dihydroxycinnamoyl-β-glucopyranosyl-(1→2)]-β-galactopyranoside) (1) as well as a new iridoid, asperulogenin in addition to eight known secondary metabolites (quercetin, kaempferol, quercetin 3-*O*-β-glucopyranosyl-(1→2)-β-galactopyranoside, quercetin 3-*O*-β-glucopyranosyl-(1→2)-arabinopyranoside, asperuloside, deacetylasperulosidic acid, asperulosidic acid methylester and chlorogenic acid). The structures of the isolates were identified by means of several spectroscopic methods including 1D- and 2D-NMR, IR and UV spectroscopy as well as mass spectrometry. This work constitutes the first phytochemical work on the title plant.

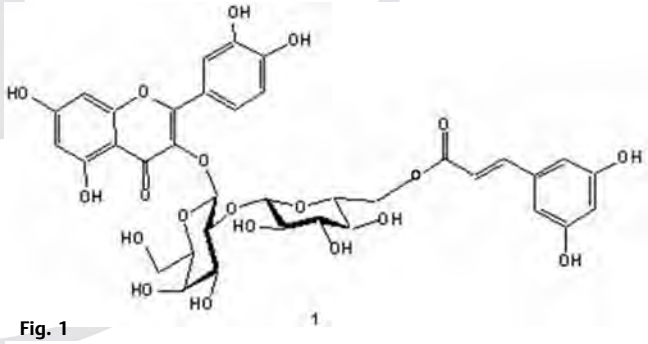

Fig. 1

References: [1] Turhan B. Therapy with medicinal plants in Turkey (Past and Present),1999, Istanbul, Nobel Tıp Kitapevi, p:371 [2] Ozgen U., Kazas C., Secen H., Coskun M. Turkish Journal of Chemistry 30 (2006): 15 – 20. [3] Tzakou O., Lempesis K., Loukis A. Natural Product Chemistry. 6 (2011): 237 – 238.

PI12 Lipase inhibitory activity and phytochemical studies of *Polygonum sericeum*

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More than 120 Mongolian plant species were screened for their *in vitro* Lipase inhibitory activity by BALB-DTNB method [1]. Among them, the stems of *Polygonum sericeum* showed significant antilipase activity. On the other hand, no reports have been published on chemistry and biological activity of this plant. *P. sericeum* Pall. Ex Georgi (Polygonaceae Juss) is an annual herbaceous plant, distributed in Khentei, Khangai, Mongol Daurian regions of Mongolia [2]. Stems of *P. sericeum* were extracted with 80% acetone. After filtration and evaporation *in* vacuo, the crude extract was suspended in water and then successively partitioned with chloroform and *n*-butanol. The *n*-butanol fraction exhibited the greatest lipase inhibitory activity with 70% at 3 mg/ml concentration. The bioactive fraction was subjected further to ODS column and HPLC, and afforded two new compounds (**1**, **2**, Figure), together with four known compounds, such as quercetin (**3**), quercetin-3-*O*-β-D-glucuronide (**4**), quercetin-3-*O*-β-D-glucuronide-6″-methyl ester (**5**) and quercetin-3-*O*-α-arabinopyranoside (**6**). All isolates are reported for the first time in *P. sericeum* by our work. Structures of the two new compounds were determined on the basis of their physico-chemical properties as well as spectroscopic analyses including ^{1}H, ^{13}C, 2D NMR and MS.

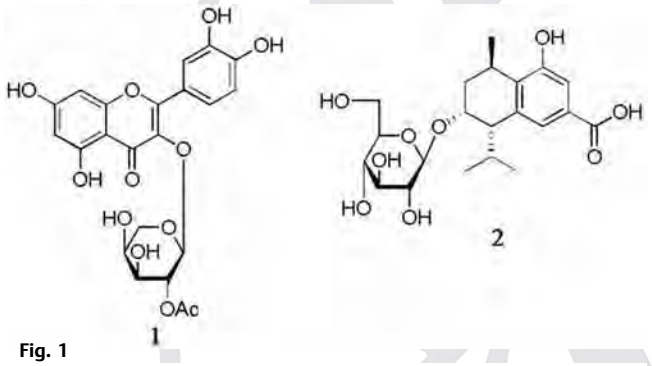

Fig. 1

Acknowledgements: *This study was partially supported by Honda Foundation, Japan.* **References:** [1] I. Furukawa et al (1982) Clinical chemistry 28/1, 110 – 113. [2] V.I. Grubov (1982). Key to the vascular plants of Mongolia (with an atlas) Nauka publishing, Leningrad, USSR.

PI13 Production of Fruticuline A and Demethylfruticuline A in *in vitro*-produced biomass from *Salvia corrugata* Vahl

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Salvia corrugata Vahl. is an American species that is cultivated in the Mediterranean coastal area as an ornamental plant. The surface exudate of the fresh aerial parts has shown a significant antibacterial activity[1,2] as well as antitumor and cytotoxic activities[3,4] due to the content of two icetaxane diterpene quinones: Fruticuline A (**1**) and Demethylfruticuline A (**2**). The extractive yield of **1** and **2**, previously isolated from *S. fruticulosa* Benth. and *S. arizonica* Gray, has been reported only for the first species (0.0026% and 0.01% (dry weight) respectively)[1]. **2** is the most abundant diterpenoid isolated from *Salvia corrugata,* where the extractive yields of **1** and **2** are 0.029% and 0.11%, respectively[1]. Aim of this work was to develop protocols for micropropagation, shoot regeneration and callus production of this species in order to evaluate the presence of **1** and **2** in the obtained biomass and to enhance the production of these compounds[5]. Shoot tips of young-herbaceous branches of *S. corrugata* have been used to start the micropropagation experiment, while stem nodes[6] and leaves were used for the experiment of induction of adventitious shoots and callus respectively. The presence of these icetaxane diterpenes have been evidenced in all the *in vitro*-obtained tissues by means of HPLC, NMR and MS experiments. **References:** [1] Bisio A, Romussi G, Russo E, Cafaggi S, Schito AM, Repetto B, De Tommasi N. 2008. *J. Agric. Food Chem*. **56**: 10468 – 10472. [2] Schito AM, Piatti G, Stauder M, Bisio A, Giacomelli E, Romussi G, Pruzzo C. 2011. *Int. J. Antimicrob. Ag*. **37**(2):129 – 34. [3] Giannoni P, Narcisi R, De Totero D, Romussi G, Quarto R, Bisio A. 2010. *Phytomedicine* **17**: 449 – 456. [4] Monticone M, Bisio A, Daga A, Giannoni P, Giaretti W, Maffei M, Pfeffer U, Romeo F, Quarto R, Romussi G, Corte G, Castagnola P. 2010. *J. Cell. Biochem*. **111**: 1149 – 1159. [5] *Alkowni* R, *Sawalha* K. *2012. Journal of Agricultural Technology,* **8**: 1285 – 1299. [6] Fraternale D, Bisio A, Ricci D. 2013. *Plant Biosystems*, in press.

PI14 Mass spectrometry guided purification for efficient isolation of natural products at semi- and preparative scale

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In natural product research the isolation of compounds from crude extracts is a key element. In this respect, the increase of the purification process' efficiency, by improvement of instrumental and methodological approaches, is a crucial point. To tackle this issue, a two-steps chromatographic strategy was developed. First, the separation of target molecules from a crude extract was optimized by application of a linear gradient at the analytical scale. Second, the gradient was geometrically transferred to semi-preparative HPLC by a gradient transfer method based on the calibration of the chromatographic systems (measurement of dwell volume and extra column volume) [1]. UV as well as MS monitoring were performed for a comprehensive detection of the various compounds present within the mixture. MS detection proved to be an important tool, not only for the purification of compounds that cannot be detected by UV, but also for an efficient mass spectrometry guided purification of specific compounds in crude extracts. In particular, a single quadrupole mass spectrometer coupled to a semi-preparative chromatographic system (PuriFlash® – MS) was found a promising tool to increase the efficiency of the isolation of constituents of interest. This MS-guided separation of compounds in complex mixtures represents a powerful strategy, not only for the isolation of molecules present in bioactive fractions, but also for the rapid purification of biomarkers identified by UHPLC-MS metabolomics and dereplication process. **References:** [1] Davy Guillarme, Dao T.T. Nguyen, Serge Rudaz, Jean-Luc Veuthey, *Eur. J. Pharma. Biopharma.* **2008**, *68*, 430.

PI15 Quantitative analysis of benzofuran neolignans from *Piper rivinoides* using nuclear magnetic resonance

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In order to characterize a sample obtained from an important medicinal plant we applied nuclear magnetic resonance to access quantitative data of its bioactive compounds. *Piper rivinoides* has been a focus of our interests due to the disclosed vasodilator activity found for its extracts and purifies compounds. Following an usual trend for *Piper* species we were able to identify four benzofuran neolignans, namely Eupomatenoid V, Eupomatenoid VI, (2R,3R)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-methyl-5-(*E*)-propenylbenzofuran and Conocarpan. In this paper we aim to characterize the bioactive fractions and the raw extract under quantitative analysis. A fundamental feature of NMR spectroscopy is that the intensity of a single resonance obtained will have direct relation with the number of spins and so with the concentration. Thus, since each of the intensity interfering parameter is optimized the integrated signal becomes directly correlated to the concentration of a certain compound. Then, key known resonances from Eupomatenoid VI (1 H, d 3JHH = 8.18 Hz, 7.66 ppm; H-6'), Eupomatenoid V (1 H, s, 7.37 ppm; H-6) and Conocarpan (1 H, d JHH = 8.10 Hz, 6.76 ppm; H-3) related to one proton each were found to be isolated, and so, capable of being quantified. The integrals of each of the selected resonances were used as their absolute values for quantitative calculations; $m^{analito} = (I^{analito}/I^{padrão}) * (MM^{analito}/MM^{padrão}) * (N^{analito}/N^{padrão}) * (1/m^{padrão})$. Dimethylbenzaldehyde resonance at 9.85 ppm (aldehyde proton, 1 H, s) was used as inter-

nal standard. The results found with basis on the equation (1) show that the evaluated extract contains 15.29% of Eupomatenoid VI, 29.81% of Eupomatenoid V, 5,52% of Conocarpan; percentages were calculated for the total powdered and dry plant material. By means of two signals from 6.15 ppm to 6.40 ppm related to two typical protons of benzofuran neolignans we could define to total benzofuran neolignans concentration in the sample.

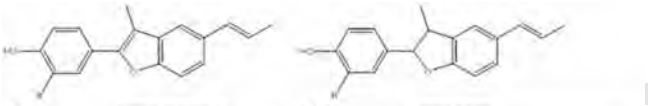

Fig. 1

PI16

Benzophenone glycosides from *Hypericum humifusum* ssp. *austral*

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The genus *Hypericum* (Hypericaceae) comprises 484 species worldwide.[1] Traditionally, *Hypericum* species and in particular *H. perforatum* have been used for the treatment of burns, rheumatism, hemorrhoids, neuralgia, gastroenteritis, ulcers, hysteria, and depression.[2] In the last decade this genus has attracted the attention of phytochemists due to the many phloroglucinols, naphthodianthrones, flavonoids, benzophenones, xanthones, and phenolic acids that have been isolated.[2] *H. humifusum* L. ssp. *austral* Rouy et Foue, is a glabrous plant, growing in Tunisia, whose length is between 10 and 40 cm.[1] The species is known for its use in Spain folk medicine as an agent treating digestive disorders and skin diseases.[3] Our previous studies reported the essential oil composition of *H. humifusum*;[4] however no previous phytochemical investigation on this species was carried out to date. In this work, carried out in the frame of a project of the Tunisian Ministry of the Higher Education, Scientific and Technology, we described the isolation and structural identification of six new benzophenones: 2,3',4,5',6-pentahydroxybenzophenone 4-*O*-(6''-benzoyl)-β-D-glucopyranoside (**1**), 2,3',4,5',6-pentahydroxybenzophenone 4-*O*-β-D-glucopyranoside (**2**), 2,3',4,5',6-pentahydroxybenzophenone 2-*O*-(2''-benzoyl)-α-L-arabinopyranoside (**3**), 2,3',4,5',6-pentahydroxybenzophenone 2-*O*-α-L-arabinopyranoside (**4**), 2,3',4,5',6-pentahydroxybenzophenone 2-*O*-(4''-acetyl)-β-D-xylopyranoside (**5**), 2,3',4,5',6-pentahydroxybenzophenone 3-*C*-(4''-benzoyl)-β-D-glucopyranoside (**6**), and five known phenolic derivatives. The structures of all compounds were determined by detailed NMR and HRESIMS analyses. **References:** [1] Crockett, S. I.; Robson N. K. B. *Plant Sci. Biotechn.* **2011**, *5*,1 – 13. [2] Borges, L. V. et al. In *Recent Progress in Medicinal Plants*; Singh V.K. Ed., **2008**, *21*, pp. 19 – 37. [3] Gonzalez-Tejero, M. R. et al. *J. Ethnopharmacol.* **2008**, *116*, 341 – 357. [4] Rouis, Z. et al. *Chem. Biodivers.* **2012**, *9*, 806 – 816.

PI17

Phytochemical study of the polar extracts of *Cenostigma macrophyllum* Tul. (Leguminosae)

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Cenostigma macrophyllum Tul. (Leguminosae) is a plant popularly known as "canela-de-velho," "caneleiro" and "catingueira" that can be found in the Brazilian "cerrado" and "caatinga" regions[1,2]. The leaves, stem barks and flowers of species of this genus are also used as folk medicines to treat stomach and intestinal disorders[3]. The aqueous leaf extract of *C. macrophyllum* showed anti-ulcer activity, and the ethanol extract presented antimicrobial, anti-inflammatory and antinociceptive activities[4]. In previous works we presented the isolation and identification of several substances with biological activity such as bergenin (antinociceptive activity), quercetin, gallic acid, ellagic acid (antioxidant activity), aurentiamide and aurentiamide acetate (anticholinesterase activity)[5,6]. This work describes the phytochemical study of the leaves of *C. macrophyllum*. The MeOH extract was partitioned between a solution of MeOH:H_2O (9:1) and CH_2Cl_2, followed by a partition with EtOAc. The EtOAc extract was submitted to a column chromatography that led to isolation and identification of the flavonoids Vitexin, Isovitexin, (2''-O-galloyl) Isovitexin and Nicotiflorin. The structures of the isolated compounds were elucidated by ^{1}H and ^{13}C NMR (uni and bidimensional), IR and MS, beyond comparison with literature dates. **Acknowledgements:** *We wish to thank CNPq and FAPESB/PRONEM (Brazil) for financial support.* **References:** [1] da Silva, M. F. Editora da Universidade Federal do Amazonas: Manaus, 2004. [2] Queiroz, L. P. Universidade Estadual de Feira de Santana: Feira de Santana, 2009. [3] Silva, H. R. et al. (2007) *Química Nova*, *30*: 1877. [4] Sousa, C. M. M. et al. (2007) *Química Nova*, *30*: 351. [5] Alves, C. Q. et al. (2012), *Química Nova*, 35: 1137 – 1140. [6] Alves, C. Q. et al. (2013), *Parmaceutical Biology*, (doi:10.3109/13880209.2013. 770536).

PI18

More triterpenoid bisdesmosidic saponins from *Agrostemma githago* L. (corn cockle)

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The common corn cockle (*Agrostemma githago* L., Caryophyllaceae) is a slender pink originally european wild-flower. Formerly widespread among wheat fields today the plant is nearly eradicated in modern agriculture and has become an endangered species that is protected by law [1]. In the past *Agrostemma githago* gained notoriety for its toxicity when poisoning people who consumed grain contaminated with the plant seeds [2]. This toxicity is believed to be a conserved defense mechanism based on a synergistic cytotoxicity of the contained saponins and the ribosome-inactivating protein type I (RIP-I) agrostin. Hebestreit et al. could approve this assumption. Both, the saponins as well as the RIP-I did only show little cytotoxicity when applied separately but revealed an increase in the RIP-I cytotoxicity in a synergistic manner when applied in combination in a cell culture model. Moreover Hebestreit et al. found an extraordinary high synergistic cytotoxicity when agrostin was combined with *Agrostemma* saponins instead of other triterpenoic saponins of similar molecular mass [3]. In 1974 Tschesche et al. and in 1998 Siepmann et al. published the structures of respectively two saponins isolated from *Agrostemma githago* L. [4, 5]. However other sources claim the isolation of further saponins, the accurate used species are usually not clear. We isolated four bisdesmosidic oleanane-type saponins with masses from 1527.8 g/mol to 1690.8 g/mol which partially enhanced the cytotoxicity of the RIP-I agrostin and saporin. **References:** [1] http://www.stiftung-naturschutz-hh.de/blume/2003.htm [2] http://de.wikipedia.org/wiki/Kornrade [3] Hebestreit, P. et al. (2006) Toxicon 47:330 – 335 [4] Tschesche, R. et al. (1974) Chem. Ber. 107:2710 – 2719 [5] Siepmann, C. et al. (1998) Planta Medica 64:159 – 164

PI19

Secondary metabolites of *Hypoxylon investiens*, an endophytic fungus derived from *Litsea akoensis* var. *chitouchiaoensis*

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Many secondary metabolites, e.g. terpenoids, polyketides, cytochalasin, tetralone, were reported on the *Hypoxylon* sp. (Xylariaceae). In this study we focus on an endophytic fungus-*Hypoxylon investiens* BCRC 10F0115, isolated from the stem of an endemic plant, *Litsea akoensis* Hayata var. *chitouchiaoensis* Liao (Lauraceae). The aim of this study was the isolation of the *n*-BuOH extract of its metabolites and the evaluation of their bioactivities. So far, three new compounds, hypoxyloamide (**1**), 8-methoxynaphthalene1,7-diol (**2**), hypoxylonol (**3**) and five compounds first isolated from nature sources, investiamide (**4**), hypoxylopropanamide (**5**), (*S*)-5-methyl-8-*O*-methylmellein (**6**), hypoxylakolone (**7**), and hypoxylakolone A (**8**), together with 16 known compounds (**9 – 24**). Among these isolates, 8-methoxy-naphthalen-1-ol (**17**) and

1,8-dimethoxy-naphthalene (**18**) showed anti-inflammatory activities to inhibit iNOS, NO, and IL-6 production in LPS-activated RAW 264.7 cell, and **17** showed the stronger NO inhibitory activity at 10 μg/ml than the positive control quercetin, with IC_{50} value of 17.8 μM without cytotoxicity.

PI20 Bioactive constituents from *Pteris ensiformis*

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Pteris ensiformis Burm. is a perennial herb, distributed in South China, India, Malay Peninsula, South Japan, Ryukyu Islands, the Philippines, Tropical Australia, and Taiwan. The genus *Pteris* (Pteridaceae) comprises about 300 species with *tropical* distribution. Various sesquiterpenes, flavonoids, benzenoids, and their derivatives are widely distributed in plants of genus *Pteris*. Many of these compounds exhibit cytotoxic, antioxidant, and antidiabetic activities. Investigation of the EtOAc-soluble fraction of the whole plants of *P. ensiformis* has led to the isolation of a new pterosin sesquiterpene, (*R*)-4-*O*-methylangolensin (**1**), along with 5 known compounds. The structure of **1** was determined through extensive 1D/2D NMR and mass-spectrometric analyses. Among the isolated compounds, (2*S*,3*S*)-13-hydroxypterosin T exhibited antitubercular activities (MIC = 6.25 μg/ml) against *Mycobacterium tuberculosis* $H_{37}Rv$ *in vitro*. **Acknowledgement:** This research was supported by grants from the National Science Council (NSC, Taiwan) (No. NSC 98 – 2320-B-127 – 001-MY3 and NSC 101 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

PI21 Garcimultiflorone G, a novel benzoylphloroglucinol derivative from *Garcinia multiflora*

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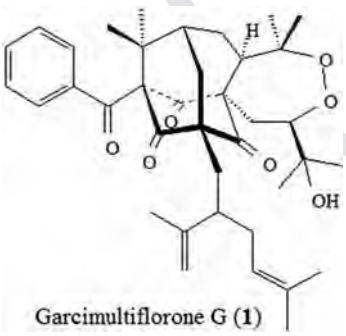
Garcinia multiflora (Guttiferae) Champ. is a small evergreen tree, distributed in South China, Hong Kong, and Taiwan. Xanthones, biflavonoids, benzophenones, phloroglucinol, and their derivatives are widely distributed in plants of genus *Garcinia*. Many of these compounds exhibit anti-inflammatory, anti-HIV, antitubercular, cytotoxic, and antioxidant activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* anti-inflammatory activity, and *G. multiflora* has been found to be an active species. In our search for compounds with anti-inflammatory activities, an additonal new benzophenone derivative, garcimultiflorone G (**1**) has been isolated and identified from the fruits of *G. multiflora*. The structural elucidation of garcimultiflorone G (**1**) was established by spectroscopic and MS analyses and its anti-inflammatory activity was evaluated by suppressing fMet-Leu-Phe/cytochalasin B (fMLP/CB)-induced superoxide anion ($O_2^{\cdot-}$) generation and elastase release by human neutrophils.



Garcimultiflorone G (**1**)

Fig. 1

Acknowledgement: This research was supported by grants from the National Science Council (NSC, Taiwan) (No. NSC 98 – 2320-B-127 – 001-MY3 and NSC 101 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

PI22 Flavonoids from the root of *Muntingia calabura* with inhibitory activity on superoxide generation and elastase release by neutrophils

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Muntingia calabura L. (Tiliaceae) is an evergreen tree, distributed in tropical America and introduced in southern Taiwan as a cultivated plant. *M. calabura* is commercially used as healthcare products for the improvement of hypertension, myocardial infarction, spasm, and inflammatory conditions. Its fruits can be processed into jam and the leaves can be used for making tea. This plant is rich in flavonoids with flavones, flavanones, flavans, and biflavans as the major constituents, some of which have demonstrated cytotoxic and anti-platelet aggregation activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for in vitro inhibitory activity on neutrophil pro-inflammatory responses, and M. calabura has been found to be an active species. Two new biflavans, (M),(2S),(2″S)-,(P),(2S),(2″S)-8,8″-dihydroxy-7,3′,4′,5′,7″,3‴,4‴,5‴-octamethoxy-5,5″-biflavan (**1**) and (M),(2S),(2″S)-,(P),(2S),(2″S)-7,8,3′,4′,5′,7″,8″, 3‴,4‴,5‴-decamethoxy-5,5″-biflavan (**2**), and a new flavone, 4′-hydroxy-7,8,3′,5′-tetramethoxyflavone (**3**) have been isolated from the root of *M. calabura*, together with 9 known compounds. The structures of these new compounds were determined through spectroscopic and MS analyses. Among the isolated compounds, 7-hydroxyflavanone exhibited potent inhibition with IC_{50} value of 4.92 ± 1.71 μM against fMLP-induced superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP). **Acknowledgement:** This research was supported by grants from the National Science Council (NSC, Taiwan) (No. NSC 98 – 2320-B-127 – 001-MY3 and NSC 101 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

PI23 New triterpenoids and anti-inflammatory constituents from *Eriobotrya japonica*

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Eriobotrya japonica (Thunb.) Lindl. (Rosaceae) is a small tree native to Japan and China that is widely cultivated for its succulent fruit. Previous studies of this plant have reported the isolation of triterpenes, sesquiterpenes, flavonoids, megastigmane glycosides, and tannins. Some of these compounds have been reported to be biologically active, exhibiting anti-inflammatory, anti-tumor, antioxidant, antifungal, anti-HIV, or hypoglycemic properties. Investigation on EtOAc-soluble fraction of the methanol extract of the leaves *E. japonica* has led to the isolation of two new triterpenoids, methyl 2α-*O*-(*E*)-*p*-methoxycinnamoyl-3β-hydroxy-urs-12-en-28-oate (**1**) and methyl 2α-*O*-(*E*)-*p*-methoxycinnamoyl-3β-hydroxy-olean-12-en-28-oate (**2**), together with 6 known compounds. The structures of two new compounds **1** and **2** were determined through spectroscopic and MS analyses. Among the isolated compounds, ursolic acid and oleanolic acid were the most effective among the isolated compounds, with IC_{50} values of 1.86 ± 0.16 and 1.48 ± 0.83 μg/ml, respectively, against fMLP-induced elastase release and superoxide generation. Our study suggests *E. japonica* and its triterpenoid derivatives (especially ursolic acid and oleanolic acid) as potential candidates for the treatment of various inflammatory diseases. **Acknowledgement:** This research was supported by grants from the National Science Council (NSC, Taiwan) (No. NSC 98 – 2320-B-127 – 001-MY3 and NSC 101 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

PI24 Bioactive constituents from the twigs of *Zanthoxylum ailanthoides*

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Zanthoxylum ailanthoides Sieb. & Zucc. (Rutaceae) is a medium-to-large-sized tree, found at low altitude in forests of China, Japan, Korea, Philippines, and Taiwan. Various benzo[*c*]phenanthridines, coumarins, lignans, flavonoids, quinolines, benzenoids, and triterpenoids are widely distributed in this plant. Many of these compounds exhibit anti-platelet aggregation, anti-HIV, and anti-inflammatory activities. Investigation on EtOAc-soluble fraction of the twigs of *Z. ailanthoides* has led to the isolation of a new benzo[*c*]phenanthridine, oxynorchlerythrine (**1**), and a new benzenoid, methyl 4-(2-hydroxy-4-methoxy-3-methyl-4-oxobutoxy)benzoate (**2**), together with 5 known compounds, including, two benzo[*c*]phenanthridines, decarine and 6-acetonyldihydrochelerythrine, and three lignans, (-)-syringaresinol, 5′,5″-didemethoxypinoresinol, and (+)-episesamin. The structure of new compound **1** and **2** were determined through spectral analyses including extensive 2D NMR data. Among the isolated compounds, decarine, (-)-syringaresinol, and xanthyletin exhibited potent inhibition (IC_{50} values ≤4.79 μg/ml) of superoxide generation by human nutrophils in response to *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB).

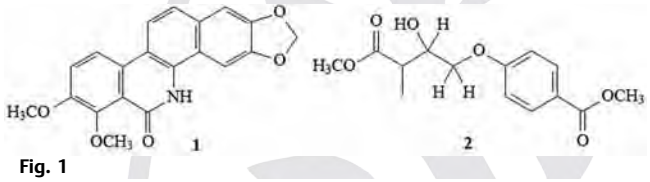

Fig. 1

Acknowledgement: This research was supported by grants from the National Science Council (NSC, Taiwan) (No. NSC 98 – 2320-B-127 – 001-MY3 and NSC 101 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

PI25 A new resveratrol derivative, 4a,4b-O-dimethyl-ε-viniferin from *Vitis thunbergii* var. *thunbergii*

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Vitis thunbergii Sieb. & Zucc. var. *thunbergii* (Vitaceae) is a liana (vine), distributed in China, Japan, Korea, and Taiwan. It has been used as a traditional medicine for diarrhea, fracture, injury, jaundice, and hepatitis in Taiwan. Various oligostilbenes, resveratrols, and their derivatives are widely distributed in plants of genus *Vitis*. In our studies on the anti-inflammatory constituents of Formosan plants and Chinese herbs, many species have been screened for *in vitro* anti-inflammatory activity, and *V. thunbergii* has been found to be an active species. Investigation on EtOAc-soluble fraction of root of *V. thunbergii* var. *thunbergii* has led to the isolation of a new resveratrol derivative, 4a,4b-*O*-dimethyl-ε-viniferin (**1**), along with 9 known compounds. The structure of new compound **1** was determined through spectroscopic and MS analyses. **Acknowledgement:** This research was supported by grants from the National Science Council (NSC, Taiwan) (No. NSC 98 – 2320-B-127 – 001-MY3 and NSC 101 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

PI26 Anti-inflammatory natural products from *Belamcanda chinensis*

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Belamcanda chinensis (L.) DC (Iridaceae) has been used as a folk medicine for the treatment of coughing and pharyngitis in China. It is effective against a number of bacterial, fungal and viral organisms and has also been used as an antidote to snakebites. In our studies on the anti-inflammatory constituents of Formosan plants and Chinese herbs, many species have been screened for *in vitro* anti-inflammatory activity, and *B. chinensis* has been found to be one of the active species. Investigation on CH_2Cl_2-soluble fraction of the rhizomes of *B. chinensis* has led to the isolation of two new isoflavone derivatives, 5-hydroxy-3′-methoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (**1**) and 5,7-dihydroxy-6,3′-dimethoxy-4′,5′-methylenedioxyisoflavone (**2**), along with 8 known compounds (**3-10**). The structures of new compounds **1** and **2** were determined through spectroscopic and MS analyses. Among the isolated compounds, isotectorigenin (**9**) exhibited potent inhibition (IC_{50}= 5.91 μg/ml) of elastase release by human neutrophils in response to fMet-Leu-Phe/Cytochalasin B. **Acknowledgement:** This research was supported by grants from the National Science Council (NSC, Taiwan) (No. NSC 98 – 2320-B-127 – 001-MY3 and NSC 101 – 2320-B-127 – 001-MY3), awarded to Prof. J.-J. Chen.

PI27 Phenolic compounds from *in vitro* cultures of *Rindera graeca* Boiss. & Feldr.

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Rindera graeca Boiss. & Heldr. (Boraginaceae) is an endemic plant of South-East Europe and Mediterranean Basin. This species, listed in the WCMC Plants Database as "Rare", grows mainly on stony slopes with shallow soils within the main range 1700 – 2200 m. To our best knowledge *R. graeca* has never been studied phytochemically before. The preliminary chemical investigation showed the presence of shikonin derivatives in the natural roots of this species cultured *in vitro*. The presence of phenolic compounds has been analyzed in established *in vitro* cultures of shoots, natural and transgenic roots of *R. graeca*. Their content of caffeic acid (CA), rosmarinic acid (RA), lithospermic acid (LA) and lithospermic acid B (LAB) has been confirmed through HPLC analyses. Moreover, in the present study the n-hexane extract of the *in vitro* natural roots and the methanolic extract of the *in vitro* cultivated shoots have been subjected to several chromatographic separations affording an unusual phenolic naphthoquinone pigment (rinderol). This new natural compound was isolated recently for the first time by our team from *Cynoglossum columnae* Ten. *in vitro* natural roots, together with two phenolic acids RA and LAB, respectively. All isolated compounds have been determined structurally by modern spectral means ($^1H/^{13}C$, ^1H-^1H and ^1H-^{13}C correlation NMR spectroscopy, ESI-MS).

PI28 Qualitative and quantitative determination of natural testosterone type steroids in pollen from two Greek *Pinus* species (*P. nigra* and *P. heldreichii*)

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European Black pine (*Pinus nigra* L.) is a widespread conifer in South Eastern Europe, while *Pinus heldreichii* Christ. var. *leucodermis* is a forest species endemic to the Balkan peninsula, often called white bark pine. It was recently referred by Buhner (1) that pollen from pines contains high percentage of testosterone (and other androgens) among plants which is in accordance with published data from early 70's and 80's confirming that pine pollen contains important quantities of steroids (2, 3). Moreover, pine pollen has been used traditionally throughout Asia and especially in China, for comparable reasons by herbalists. In the Greek market as well as worldwide, there are food supplements, based on pine pollen, recommended for male's diets, claiming of increasing muscular mass and the potential of high content in androsteroids. Due to this commercial uses, the aim of this study was the qualitative and quantitative determination of steroids in pollen from the species *Pinus nigra* and *Pinus heldreichii*, growing wild in Greece as to our knowledge they have not been studied previously. The results showed that *Pinus nigra* contains epitestosterone, 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol and etiocholanolone which are referred for the first time in

this species, while total content of steroids 1.2 μg/10gr, amount comparable with existing bibliographic data (1). *Pinus heldreichii,* not previously studied, contained the same steroids at a much higher total content of 7.57 μg/10gr of pollen. **References:** [1] Buhner S. 2007. The natural testosterone plan. Healing Art Press, Rochester, Vermont. [2] Saden-Krehula M, Tajic M, Kolbah D. 1971. Testosterone, Epitestosterone and Androstenedione in the Pollen of Scotch Pine *P. siivestris L.* Experientia 27, 108 [3] Saden-Krehula M, Tajic M, Kolbah D. 1979. Sex hormones and corticosteroids in pollen of *Pinus nigra.* Phytochemistry 18, 345 – 346.

PI29 Isolation of pyrrolizidine alkaloids from *Cynoglossum columnae* Ten. (Boraginaceae)

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Cynoglossum species such as *C. officinale*, *C. amabile* and *C. creticum*, among other Boraginaceae plants, are known to accumulate pyrrolizidine alkaloids (PAs) as a major means of chemical defense [1]. In the present study one novel PA of the C-7-*O*, 9-*O* acyclic diester type has been isolated, having the skeletal formula of 7*O*-tiglic-9*O*-(2-deoxy-2-methyl-echimidinic) diester of heliotridine N-oxide **1**, from the endemic Greek plant *C. columnae*. The methanolic extract of the aerial parts has been submitted directly to column chromatography (without reduction) and the collected fractions further subjected to prep-TLC. PA structure has been identified by means of one dimensional $^{1}H/^{13}C$, ^{1}H-^{1}H and ^{1}H-^{13}C correlation NMR spectroscopy, while ESI-MS verified the calculated molecular weight. The absolute configuration of the necine base as heliotridine was deduced according to observed NOESY correlations, while the stereochemical structure of the 9-*O*-esterified necic acid was not determined. Moreover, three known pyrrolizidine alkaloids (PAs) were also isolated from *C. columnae*, namely the *N*-oxides of echinatine, rinderine and 3'-O-acetylrinderine.

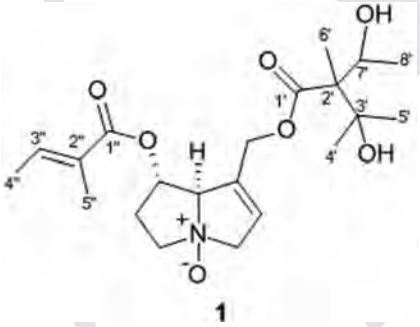

Fig. 1

References: [1] El-Shazly, A. et al.(1996) Biochem. System. Ecol.24:415 – 421.

PI30 HS-SPME-GC/MS chemical analyses of fresh black Truffles growing in Greece. Biological activities

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Mushrooms are a popular food, due to their unique flavour and taste, nutritional properties and health benefits. Among all edible mushrooms, the genus *Tuber* is the most well known for truffles, as it has a number of distinctive characteristics and high economic value [1]. Among *Tuber* species, winter black truffles (*Tuber melanosporum*) and black summer truffles (*T. aestivum*) are among the most highly prized in France, Italian and Greek cuisines. The black truffles have a long history as they were known in Ancient Greece for their aphrodisiac activities, described by Theophrastus, Dioscoridis and Galen as "idna" (δνα). To our knowledge, both the winter and summer black truffles, growing in Greece, have never been studied before. In this study, fresh samples from Greek black truffles (*T. melanosporum* and *T aestivum*) have been studied for their volatiles through head-space solid-phase micro-extraction combined with GC-MS analyses (SPME-GC/MS), while their extracts have been submitted to several classic chromatographic procedures. Several sterols such as ergosta-5,7,22-trien-3β-ol, brassicasterol (ergosta-5,22-dien-3β-ol) and stigmasterol were identified and isolated as well as the volatile organic compounds (VOCs): hexadecene and 3-penten-2-one together with other VOCs such as: 2-methyl butanal, 3-methyl butanal, benzophenone as well dimethyl sulfide and disulfides. Moreover, the ethanolic extracts of the truffles were assayed for their antioxidative activity using Rancimat technique showings high activities, while they were also tested for their antimicrobial properties against a panel of human pathogenic microorganisms showing an interesting profile, as *T. melanosporum* expressed strong antibacterila activity (MIC values 0.44 – 1.45 mg/ml) while *T. aestivum* appeared less active. **References:** [1] Wang S. & Marcone M. The biochemistry and biological properties of the world's most expensive underground edible mushroom:truffles" Food res. International 2567 – 2581 (2011).

PI31 Analysis of the flavonoid compounds and antioxidant capacity of the leaf extract obtained from *Arrabidaea chica* cultivated in Southern Brazil

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There is a growing interest in natural antioxidants present in medicinal and dietary plants that might help attenuate oxidative damage. To identify antioxidant from herbs, we have investigated the antioxidant potential from *Arrabidaea chica* (HBK) Verlot). This plant has been used as an anti-inflammatory and astringent agent as well as remedies for intestinal colic, diarrhea, leucorrhoea, anemia and leukemia. This study aimed to develop a high-performance liquid chromatographic (HPLC) method for the validation and quantification of scutellarein and apigenin obtained from the extract of the leaves of *Arrabidaea chica* and to analyze the antioxidant activity of the crude extract using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, β-carotene bleaching test, and total reactive antioxidant potential (TRAP) test. The validation of the HPLC method using apigenin and scutellarein as external standards showed that the method was precise and accurate and afforded good linearity. The method allowed the identification and quantification of apigenin (112.04 ± 0.52 μg/mL) and scutellarein (818.60 ± 3.26 μg/mL) in the extract of *A. chica.* The crude extract quenched DPPH free radicals in a dose-dependent manner and the IC_{50} of the extract was 13.51 μg/mL. The β-carotene bleaching test showed that the addition of the *A. chica* extract in different concentrations (200 and 500 μg/mL) prevented the bleaching of β-carotene at different degrees (51.2 ± 3.38 and 94 ± 4.61 %). The TRAP test showed dose-dependent correlation between the increasing concentrations of *A. chica* extract (0.1, 0.5 and 1.0 μg/mL) and the TRAP values obtained by trolox (hydro-soluble vitamin E) that were 0.4738 ± 0.0466, 1.981 ± 0.1603 and 6.877 ± 1.445 μM Trolox respectively. The results of these tests showed that the extract of *A. chica* had a significant antioxidant activity, which could be attributed to the presence of the mixture of flavonoids and others compounds in the plant extract.

PI32 Chemical phytoconstituents of *Amyris pinnata*

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Amyris is a plant genus usually very aromatic. Some species have been used for timber and as a source of incense and oil with several attributed medicinal properties. As part of our research on Colombian Rutaceae plants, a phytochemical exploration was carried out on *Amyris pinnata* Kunth (bark and leaves), as well as the antitumor activity evaluation on P-388, M-17 and H-116 tumor cell lines. So far, only a work had described the isolation and the cytotoxic activity of five coumarins and three butyrolactone lignans from *A. pinnata* leading to the identification of the cytotoxic lignan austrobailignan-1. Herein described is the

isolation of six lignans, three coumarins, a sesquiterpene, an oxazole alkaloid and a prenylated flavonoid, whose chemical structures were established by fully analyses of spectroscopic data (1D and 2D NMR, MS) in comparison with data in the literature. All compounds exhibited antitumor activity at different levels. Butyrolactone and aryltetralin lignans exhibited the best cytotoxic activity (ED_{50}= 2.0 – 20 μM) against cell lines. Sesquiterpene was only active against H-116 cell line. Remaining test compounds exhibiting low cytotoxic activity.

PI33 Chemical composition, cancer chemopreventive and acetylcholinesterase activities of *Spondias tuberosa*

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The Brazilian biodiversity is a source of great variety of edible fruits used as food by the local population. In most cases, these fruits have only been poorly studied for their chemical composition and biological activity. The purpose of this study was to evaluate the chemical composition of the pulp of Umbú (*Spondias tuberosa* Arr. Camara, Anacardiaceae) followed by the analysis of cancer chemopreventive and acetylcholinesterase inhibition activities. At 40 μg/ml, the methanolic extract presented high antioxidant activities in the DPPH (89%), ABTS (97%) and ORAC (64%) assays, as well as 61% inhibition of acetylcholinesterase activity. The dichloromethane extract exhibited quinone reductase induction with an induction ration of 2.8 at 20 μg/ml in Hepa1c1c7 cells. The dichloromethane extract was analysed by normal phase HPLC and methanolic one carried out in C18 phase. The bioassay-guided isolation was undertaken following these various biological activities by using HPLC-microfractionation in 96 well plates and biological assays to localize the active compounds from the HPLC chromatogram in both extracts. The analytical HPLC conditions were geometrically transferred to a preparative medium pressure liquid chromatography (MPLC) by chromatographic calculations. This method was used to isolate the active compounds from the methanolic extract. All of the MPLC fractions were monitored by UHPLC/TOF/MS provide a 2D LCxLC plot of the MPLC separation. Six compounds were isolated for the first time in this plant in a single step and a new compound derived from gallic acid was found. The structures of the isolated compounds were elucidated by classical spectroscopic methods including 2D NMR and HR-MS. Such type of studies may lead to the development of functional foods with valuable health benefits and/or may provide new natural products with interesting potential activities against anti-aging diseases.

PI34 *Copaifera langsdorffii* aqueous extract and its galloylquinic acid display gastroprotective activity

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Ulcer is a result of the imbalance between aggressive and protective factors in the stomach tissue. *Copaifera langsdorffii* Desf. is a tree that grows in Brazil and its oleo-resin has great potential for the treatment of gastric disorders based on the traditional use[1]. The aim of this study was to investigate the antiulcer activities of *C. langsdorffii* aqueous extract (**AE**) and its galloylquinic acid (**GQ**) compound. For that, the hydroalcoholic extract was partitioned with organic solvents and the remaining **AE** was lyophilized. **AE** was then submitted to Sephadex LH-20 using a gradient of methanol:water (1:9 to 9:1). A **GQ** was isolated from the Sephadex fractions by reverse phase high performance liquid chromatography (HPLC-RP). The compound was identified by nuclear magnetic resonance (NMR) means. The gastroprotective activity was evaluated using Ethanol/HCl induced ulcer model[2]. *Balb-C* male mice (n = 6, 20 ± 3 g) were orally treated with 30, 100 and 300 mg/kg of **AE**, and 30 mg/kg of **GQ**. Pantoprazole (30 mg/kg) and saline solution (0.9%) were used as controls. One hour after the treatment, animals received Ethanol/HCl (60%/0.03N) to induce stomach lesions, and one hour later animals were euthanized and lesions were analyzed. In the total extract, the **AE** corresponded to 28.7%. The **GQ** was isolated after collecting 411 fractions (20 mL) from Sephadex column, which was further purificated by HPLC-RP. The **AE** exhibited gastroprotective activities of 79%, 84% and 88%, at doses of 30, 100 and 300 mg/kg, respectively. Pantoprazole and **GQ** displayed 93.1% and 93.8% of gastroprotective activities, respectively. Therefore, the rich galloylquinic **AE** and its isolated **GQ** displayed remarkable gastroprotective effects. However, further studies are needed to confirm the mechanisms involved in the gastroprotection. **Acknowledgment:** FAPESP **References:** [1] PAIVA, *et al.* J Ethnopharmacol 62 (1998) 73 – 78. [2] MIZUI & DOTEUCHI Jpn J Pharmacol 33, (1983) 939 – 945.

PI35 Identification of the ellagitannin geraniin as a novel Hsp90 inhibitor

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Heat shock protein 90 (Hsp90) is a highly conserved molecular chaperone assisting the proper protein folding and assembly, and targeting misfolded proteins to the proteolytic degradation.[1 – 3] Inhibition of the Hsp90 activity incapacitates simultaneously multiple client proteins, resulting in a blockade of multiple signaling pathways and, ultimately, providing a combinatorial attack to cellular oncogenic processes.[4] Because of the potential therapeutic use in multiple cancer indications, Hsp90 has emerged as an exciting new target for the development of antitumor agents. In an effort to discover new small molecules able to inhibit the Hsp90 ATPase and chaperoning activities, we screened, by a surface plasmon resonance assay, a small library including different plant polyphenols. The ellagitannin geraniin, was identified as the most promising molecule, showing a binding affinity to Hsp90 similar to 17-(allylamino)-17-demethoxygeldanamycin (17AGG), one of the most potent known anti-Hsp90 agent. Geraniin was found to inhibit in a dose–dependent manner the Hsp90 ATPase activity, with an inhibitory efficiency similar to that measured for 17-AAG. In addition, this compound compromised the chaperone activity of Hsp90, monitored by the citrate synthase thermal induced aggregation. We also proved that following exposure to different concentrations of geraniin, the level of expression of the client proteins c-Raf, pAkt, EGFR was strongly down–regulated in HeLa and Jurkat cell lines. These results, along with the finding that geraniin did not exert any appreciable cytotoxicity on normal cells, encourage further studies on this compound as a promising chemical scaffold for the design of new Hsp90 inhibitors. **References:** [1] Pratt W.B,. et al. *Exp Biol Med.* **2003**, *228*, 111 – 133. [2] Zhang H. et al. *J Mol Med.* **2004**, *82*, 488 – 499. [3] Zhang T. et al. *Mol Cancer Ther.* **2008** *7*, 162 – 170. [4] Trepel J. et al. *Nat Rev Cancer.* **2010**, *10*, 537 – 549.

PI36 *In vitro* cytotoxic activity of essential oil from khaya species flowers

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The essential oil from fresh flowers of *Khaya grandifoliola* C.DC and. *Khaya senegalensis* A. Juss were obtained by hydro distillation method, in a modified Likens and Nickerson apparatus which allowed the distillation and simultaneous extraction of the volatile components in an organic solvent (n-pentane). The n-pentane layer was collected, filtered over anhydrous sodium sulfate, cautiously evaporated. A content of 0.09 – 0.1% essential oil was established. The chemical composition of the essential oil was analyzed by gas-chromatography coupled with mass spectrometry. Sesquiterpenes represents (88%) of the identified compounds. The main compounds identified in essential oil were: caryophyllene-oxide (23.01%), isocaryophyllene, (Z) (21.99%), α- Humulene (13.46%) in *K. grandifoliola*. while: caryophyllene-oxide (14.85%), trans-Caryophyllene (43.59%), α- Humulene (15.72%) in *K. senegalensis*. The oils were tested for cytotoxic activity against HepG-2, MCF-7 and HCT-116 cell lines. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT using Doxorubicin as reference drug. *K. grandifoliola* and *K. senegalensis* oil displayed cytotoxic activity with LC_{50} (21.6, 26.1, 37.6 ppm) and (61.1, 79.7, 61 ppm) respectively. Sesquiterpene content of the oils may contribute to their cytotoxic activity. This is the first study for oil from *khaya* species.

PI37 Secondary metabolites of *Plantago lagopus* L.

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The genus *Plantago* (Plantaginaceae) is represented by 21 species in Turkey (1). Up to now, several effects have been described for the genus *Plantago* such as antitumoral, antiinflammatory, antibacterial, analgesic and hepatoprotective (2,3). Earlier investigations about *Plantago* species resulted in the isolation of mainly iridoid, phenylethanoid and flavonoid glycosides; polysaccharides and lipids (4). In this study; as a result of bioactivity guided isolation method, acteoside and calceorioside A were isolated from the most active fraction and they showed strong radical scavenging effect against DPPH, NO and SO radicals and cytotoxic activity against HEP-2, RD and MCF-7 cancer cell lines. In addition; aucubin, catalpol, rehmaglutine D and phlomuroside were obtained from the inactive fractions. Isolation of rehmaglutine D and phlomuroside from *P. lagopus* is important for the chemotaxonomic view of the genus. This is the first report for the bioactivity guided isolation of acteoside and calceorioside A from *P. lagopus*. This study was supported by the project of TUBITAK (108T518). **References:** [1] Davis, P.H. Flora of Turkey and the East Aegean Islands (1982). [2] Baytop, T. Therapy with Medicinal Plants in Turkey (Past and Present) (1999). [3] Stanisavljevic, I.T., et al., Separation Science and Technology (2008). [4] Samuelsen, A.B., Journal of Ethnopharmacology (2000).

PI38 Study of intestinal permeability of the major secondary metabolites present in the hydroalcoholic extract from the leaves of *Lychnophora salicifolia* using the human cell line Caco-2

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Lychnophora salicifolia Mart. (Asteraceae, Vernonieae) is an endemic plant from the Brazilian Cerrado known as "arnicão". It is popularly known to have anti-inflammatory and analgesic activities. Furthermore, the leaves of *Lychnopora sp.* are used as flavorings for the Brazilian traditional spirit "cachaça". Because of the limited knowledge on the bioavailability of phytochemicals from plants used in Brazilian folk medicine, in this work, we studied the possible permeation in Caco-2 cells of the major components of hydroalcoholic extracts prepared from the leaves of *L. salicifolia:* vicenin-2 and lychnopholic acid. We observed that the *C*-glucoside vicenin-2 was not transported, suggesting no absorption and no efflux of this compound in this model. However, the sesquiterpen lychopholic acid crossed the Caco-2 cells monolayer by passive diffusion and might have high absorption rate *in vivo.*

PI39 Metabolomic studies on *Isatis tinctoria* - Comparison of different origins, harvesting dates, and the effect of repeated harvesting

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Isatis tinctoria (Brassicaceae) is an ancient dye and medicinal plant with potent anti-inflammatory and anti-allergic properties [1]. We investigated metabolic differences by NMR spectroscopy of plants grown under identical conditions on experimental plots at the Agricultural Field Station of Thuringia in Dornburg, Germany. Comparisons were carried out for plants of different geographic origins, different harvesting dates, and between single and repeatedly harvested plants. For the study, plants of six origins were compared, and they were harvested at six time points. In addition, the effect of repeated harvesting was investigated. Leaf samples were shock-frozen with liquid N_2 immediately after harvest, freeze-dried, and cryomilled prior to extraction. Extracts were prepared by pressurized liquid extraction (PLE) with 70% aqueous methanol. The spectra were analyzed by multivariate data analysis. The score plots produced by principal component analysis (PCA) revealed differences in the metabolic profile between the harvesting dates. The loading plots showed that the spectral region of carbohydrate resonances was responsible for these differences. In contrast, no major differences were seen in the metabolites of different origins. Partial least square discriminant analysis (PLS-DA) revealed no effect of repeated harvesting on the metabolic profiles. **Reference:** [1] Hamburger M., Phytochemistry Reviews, 1, 333 – 344 (2002)

PI40 Expression of cardenolide-biosynthetic genes under stress conditions

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Cardenolides, like many other small plant natural products, may play a role in plant defense by acting as deterrents to herbivores (Berglund & Ohlsson, 1992). As for humans cardenolides have a long tradition in the therapy of cardiac insufficiency (Mutschler, 2008). New therapeutic roles for cardenolides in various diseases are discussed. Among them, the susceptibility of cancer cells to cardenolides in tumor therapy is of special interest (Newman et al., 2008). Cardenolides are still isolated from plants since their structural complexity impede their chemical synthesis. Their biosynthesis is affected by phytohormones, abiotic and biotic stress (Pérez-Bermúdez et al., 2009). Improving cardenolide production requires a detailed knowledge of their biosynthesis. To understand the regulation of genes encoding enzymes involved in cardenolide biosynthesis their differential expression under stress conditions was investigated in two different plant species, namely *Digitalis lanata* and *Erysimum crepidifolium*. Over decades cardenolide biosynthesis was mainly investigated in *Digitalis*. Therefore, we started our investigations with *D. lanata* shoot cultures. Stress was induced by permanent darkness and addition of bioactive enones. The expression of several genes was monitored, namely *progesterone 5*β*-reductases 1* and *2 (P5*β*R,P5*β*R2), 3*β*-hydroxysteroiddehydrogenase 1 (3*β*HSD1)* and *actin* (as a control). All genes showed a constant expression except for *P5*β*R2*, which was subject to fluctuations and inducable by bioactive enones. Because of its close relationship to *Arabidopsis thaliana*, *Erysimum crepidifolium* was established as a new model plant to study cardenolide biosynthesis (Munkert et al., 2011). *E. crepidifolium* shoot cultures were established and cardenolide formation as well as differential gene expression after stress induction of the genes mentioned above were also investigated in the new system.

PI41 Isolation of some chemical constituents from licorice and their evaluation as anticancer and antiviral agents

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Ten compounds were isolated from the methanol extract of *Glycyrrhiza glabra L.*, they were identified as 2-O-β-D-Xylopyranoside,(3- methoxy phenyl) 4'-methoxy, 3',5' dihydroxybenzofuran [1], ergosta-7,22-dien-3β,5α,6β,9α-tetraol [2], 5,2',4'-trihydroxy, 8,3'-dimethoxy 4',5' hydroxyisopropyldihydrofuran flavanol [3], isolicoflavanol [4], 7-O-β-D-xylopyranosyl (1→4) β-D-glucopyranosyl 6-O-isobutyl isoflavone [5], licorice saponin L 3 [6], Lipinifolin [7], licochalcone B [8] isoliquirtin [9] and isoliquiritigenin [10]. Eight compounds were evaluated for anticancer and antiviral activities, they were tested on three cytokines of the human ascites fluid, TNF-alpha, Interferons-gamma and NO. All tested compounds showed significant curative effect compared to control. Compound [8] showed significant decrease compared with control ($p < 0.01$) in both HCV and malignant HCV cases in the three cytokines of the human ascites fluid after 24 and 48 hours followed by compound [3].

PI42 Hepatoprotective activity of *Brassica oleraceae* L. var. *italica*

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Three compounds were isolated from ethanol extract of the powdered inflorescences of *Brassica oleracea* L. var. *italica* Plenk by special methods. These compounds were identified through spectroscopic analysis as obtucarbamate, N-(4-hydroxyphenyl) acetamide and p-hydroxybenzoic acid. When total ethanol and different successive extracts were investigated for hepatoprotective activity [1] (prophylaxis and therapeutic), the biochemical parameters revealed that, all the extracts showed significant decrease in the liver enzymes when paracetamol causes liver damage was given before the extract (therapeutic) or given after the extract (prophylactic). By scrutinizing the histopathological [2] results, it is obvious that the prophylactic activity of the extracts was better than the curative effect. In case of pretreatment of rats with different extracts (1 g/kg) for seven days prior to a single dose of paracetamol (2 g/kg) that most of the hepatic lobules appeared more or less like normal especially petroleum ether extract, ethyl acetate combined with ethanol extract and chloroform extract which assure the results obtained from the biochemical parameters. As for the rats that were given a single dose of paracetamol (2 g/kg) followed by the extracts (1 g/kg) for seven days, the tested extracts showed swelling, degeneration in the hepatocytes and some other rats showed congestion in the central vein. In addition, liver of other rats showed normal structure. So it can be concluded that broccoli has a good activity as hepatoprotective against liver damage.
Keywords. *Brassica oleracea*, obtucarbamate, hepatoprotective. **References:** [1] Mitra S. K., Venkataranganna, M. V., Sundaram R. and Gopumadhavan, S. (1998). Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. Journal of Ethnopharmacology, 63(3): 181 – 186. [2] Drury, R.A.B. and Wallington E.A. (1980). Carleton's histopathological technique, 4th edition, Oxford University Press.

PI43 Two Egyptian herbal medicines, *Tanacetum sinaicum* and *Pulicaria undulata* with nitric oxide production inhibitory effects in RAW264.7 macrophage cells

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The methylene chloride/methanol (1:1) extracts from the air-dried aerial parts of wild *Tanacetum sinaicum and Pulicaria undulata* collected in North Sinia, Egypt, showed inhibitory effects on lipopolysaccharide (LPS)-induced nitric oxide (NO) in RAW264.7 macrophage cells. Five new sesquiterpenes were isolated from these two species; compounds (**1**-**3**) and (**15**-**16**) from *T. sinaicum and P. undulata, respectively;* together with twenty five known sesquiterpenes from both species. Structures were elucidated by employing extensive 1D, 2D NMR and HR-FAB-MS experiments. In addition, all isolated sesquiterpenoids significantly inhibited the production of NO. Particularly, 3-methoxytanapartholide (**4**, IC_{50}= 1.0 *m*M), Iivalin (**17**, IC_{50}= 2.0 *m*M) and 2*a*-hydroxyalantolactone (**18**, 1.8 *m*M) were found to show stronger inhibitory effects.

PI44 Isolation and structure elucidation of new myrsinane-type diterpene polyesters from *Euphorbia falcata* L.

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Diterpenes occurring in plants of the Euphorbiaceae family are of considerable interest as concerns natural product drug discovery programs because of the wide range of potentially valuable biological activities and the broad structural diversity due to the different polycyclic and macrocyclic skeletons and various aliphatic and aromatic ester groups. In continuation of our investigations on diterpene content of *Euphorbia falcata*, ten compounds were isolated from the chloroform-soluble fraction of the MeOH extract of the plant. The present paper reports the isolation and structure determination of these diterpenes. The selected fractions were purified by RPC and preparative TLC to yield pure compounds. The structure elucidation was performed by extensive spectroscopic analysis, including 1D and 2D NMR (^{1}H-^{1}H COSY, HSQC, and HMBC), and HRESIMS experiments. The isolated compounds contain myrsinane, premyrsinane and cyclomyrsinane skeletons, esterified with acetic, propanoic, 2-methylbutanoic, isobutanoic, benzoic and nicotinic acids. Nine of them are new natural products and one is known (euphorprolitherin D) compound, isolated previously from the Asian species, *E. prolifera*. The compounds reported here are biogenetically related to those previously described from this species by our group. On the basis of the literature data, only *E. falcata,* and *E. seguieriana* has the unique diterpene profile, containing myrsinane, premyrsinane and cyclomyrsinane-type compounds together. **Acknowledgements:** This work was supported by the European Union and co-funded by the European Social Fund TÁMOP-4.2.2.A-11/1/KONV-2012 – 0035.

PI45 Chemical composition of essential oils of *Grindelia squarrosa* (Pursh) Dunal and *Grindelia oregana* A. Gray

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The genus *Grindelia* (Asteraceae) comprises about 60 species. All are native to North and Central America and chiefly distributed in warm-temperate regions. Several species are cultivated as ornamentals in Europe. Grindeliae herba traditionally is used as an adjuvant for treatment of catarrhs of upper respiratory tract, antispasmodic, expectorant. The essential oil of *Grindelia squarrosa* (Pursh) Dunal and *Grindelia oregana* A. Gray cultivated in Romania was isolated by hydrodistillation. The essential oils were analysed by combination of GC and GC/MS. The identification of the constituents was achieved from their retention indices and comparison of their MS data with computer library database and with literature data. The fifty identified constituents accounted 72.1 – 81.3% of the oils. These oils were found to contain α-pinene, β-pinene, limonene, borneol, bornyl acetate and germacrene D as main constituents. The oils obtained from the two species showed small differences in chemical composition. However, menthol, menthone and pulegone were detected only in the essential oil of *G. oregano*. **Acknowledgements:** The presentation is supported by the European Union and co-funded by the European Social Fund (TÁMOP-4.2.2.A-11/1/KONV-2012 – 0035).

PI46 Flavonoids from *Neurada procumbens* L. (Neuradaceae) in Egypt

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Seven flavonoids; taxifolin (**1**), taxifolin 3-*O*-β-rhamnopyranoside (**2**), vitexin (**3**), vitexin 2'-*O*-α-rhamnopyranoside (**4**), orientin 2'-*O*-α- rhamnopyranoside (**5**) and isoorientin 2'-*O*-α-rhamnopyranoside (**6**), *p*-hydroxybenzoic acid (**7**) have been isolated from the whole plant of *Neurada procumbens* L. Antioxidant and cytotoxicity properties of the methanol and water extracts were investigated. The chemotaxonomic significance was also investigated.

PI48 Isolation of a natural herbicidal constituent from culture filtrates of *Drechslera hawaiiensis* for management of *Rumex dentatus*

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Rumex dentatus is a problematic broad-leaf weed of wheat in Pakistan. The present study was carried out to identify natural herbicidal constituents from culture filtrates of a phytopathogenic fungus *Drechslera hawaiiensis* for the management of *R. dentatus.* Culture filtrates of the fungus prepared in M-1-D broth, significantly reduced germination and seedling growth of *R. dentatus* in a laboratory bioassay conducted in 9-cm diameter Petri plates. Fungal culture filtrates were extracted successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Bioassays were carried out on punctured leaf discs of the weed using different concentrations (2, 1, ..., 0.0625 mg mL^{-1}) of the crude organic solvent fractions. Chloroform fraction exhibited the best herbicidal activity in terms of necrotic spot formation and leaf discolouration. Five compounds from chloroform fraction were separated and purified by preparative thin layer chromatography followed by reversed phase high performance liquid chromatography (HPLC). Efficacy of different concentrations (2, 1, ..., 0.03125 µg $µL^{-1}$) of the isolated compounds was assessed by leaf disc bioassays using a well known synthetic herbicide 2 – 4, D as reference. Structure of compound with the highest herbicidal activity against*R. dentatus* was determined by various spectroscopic techniques viz. NMR and MS. The compound was identified as (Z)-docos-5-en-1- acid. This is the first report of this herbicidal compound from genus *Drechslera.*

PI49 Chemometric analysis on *Acanthopanax* fruits using UPLC-ESI-MS

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Acanthopanax species are indigenous medicinal plant and the fruits of *Acanthopanax* species have been used as a remedy for "wipe out evil wind" in traditional oriental medicine. Although there have been a few phytochemical studies on Acanthopanax fruits, no study on the comparison of anthocyanin contents and types between *Acanthopanax* species distributed in Korea, was performed. By using UPLC profiling and ESI-MS profiling of the extract of *Acanthopanax* fruit, chlorogenic acid, syringin, delphinidin lathyroside, cyanidin lathyroside, hyperoside and dicaffeoylquinic acid were identified and major components. In order to develop a new chemometric classification method using UPLC fingerprint for Acanthopanax fruits and provide a platform for the application to the discrimination of species-specific difference, 9 species of *Acanthopanax* fruits were selected. *Acanthopanax divaricatus* var. *albeofructus*, *A. divaricatus* f. *distigmatis*, *A. divaricatus* f. *flav-flos*, *A. sessiliflorus*, *A. seoulense*, *A. senticosus* f. *inermis*, *A. gracillstylus*, *A. koreanum* and *A. trifoliatus* f. *spinofolia* were chosen for this study and nine sample of each species were picked from Kyung Hee university botanical garden. Extract of each sample and partial-least squares method in discriminate analysis (PLS-DA) was performed as multivariate analysis. The extracts of *Acanthopanax* species were successfully discriminated from each species according to their characterizing UPLC-ESI-MS fingerprint and cyanidin lathyroside and chlorogenic acid were identified as major marker molecules.

PI50 Solid state fermentation of the winter savory (*Satureja montana* L) plant: changes of chemical composition and antimicrobial activity

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Solid State Fermentation (SSF), which consists of the microbial growth and product formation on solid particles in the absence of an aqueous phase, has recently attracted special attention of researchers. The aim of this study was to evaluate the chemical composition and antimicrobial activity of winter savory plant fermented with lactic acid bacteria (LAB) by using SSF. Dried winter savory plant was fermented under SSF conditions for 72 hours using *Lactobacillus* and *Pediococcus* genera belonging to isolated LAB from spontaneous Lithuanian rye sourdoughs. The essential oils were extracted using supercritical carbon dioxide and the composition of volatile compounds was analyzed by means of GC-MS. The antimicrobial activity of extracts was tested using an agar well diffusion assay method. The main compounds found in the headspace of the winter savory plant were carvacrol 38.06%, thymoquinone 10.92%, thymol methyl ether 8.17%, p-cymene 7.54%, caryophyllene 7.29%, whereas in fermented products the main compound was carvacrol. The amount of this compound increased up to 61.28 – 72.13%. After fermentation the relative amounts of α-terpineol, thymol, carvacrol, β-elemene, δ-cadinene, caryophyllene oxide and cadin-4-en-10-ol in the supercritical carbon dioxide extracts were increased. After fermentation with *P. pentosaceus* new compounds were determined: β-selinene, α-muurolene, β-farnesene and cubebol. Moreover after fermentation with *P. acidilactici* p-cymen-8-ol, tetrahydrofurfuryl alcohol, naphth-1-ol and n-tetradecane were determined. Essential oils extracts from the fermented winter savory products showed a higher antimicrobial activity against *Pseudomonas* spp. strains and *B. subtilis* in comparison with the non-fermented plant. The study highlights the possibility to use SSF for developing natural flavored products that besides flavoring have an antimicrobial effect. The research was funded by a grant (project SVE-09/2011 BIOFITAS) from the Research Council of Lithuania.

PI51 Antioxidant activity and polyphenol content of methanol extracts from *Arisarum vulgare*

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Arisarum vulgare is generally known as a toxic plant but it has some medicinal uses. The plant collected from National Park of El-Kala (north-east of Algeria), is phytochemically screened [1], and the total phenolic [2] and flavonoids [3] compounds were measured for its methanolic extract. In addition, the antioxidant capacity of the methanolic extract of this plant is evaluated by ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) tests expressed by vitamin C Equivalent Antioxidant Capacity (VCEAC) [4]. The VCEAC values are 0.3472 g and 0.2768 g of VCEAC/100 g of dry weight; as determined by ABTS and DPPH tests respectively. The total phenolic and total flavonoids contents are, respectively: 2.138 g of gallic acid equivalent/100 g of dry weight, and 7.126 g of quercetin equivalents/100 g of dry weight. A direct correlation between phenolic compounds and antioxidant activity was observed (R^2 = 0.95). According to the results, it is observed that the *Arisarum vulgare* possess a considerable antioxidant and antiradical capacity, therefore the antioxidant properties might increase the therapeutic value of this medicinal plant. **Key words:** *Arisarum vulgare*, antioxidant, polyphenols, flavonoids, ABTS, DPPH. **References:** [1] Harbone, JB, 1984. Phytochemical methods. A guide to modern techniques of plants analysis, Chapman & Hall, 2nd ed. London (1984). [2] Amarowicz R., Estrella I., Hernandez T., Troszynska A., 2008. Antioxidant activity of extract of Adzuki bean and its fractions. J. Food Lipid. 15, 119 – 136. [3] Chun O.K., Kim D.O., Lee C.Y., 2003. Superoxide radical scavenging activity of the major polyphenols in fresh plums. J. Agric. Food Chem. 51, 8067 – 8072. [4] Djilani A, Toudert N and Djilani SE. Evaluation of the Hypoglycemic Effect and Antioxidant Activity of

Methanol Extract of *Ampelodesma mauritanica* Roots. *Life Sciences and Medicine Research* (2011).

PI52 Comparative pharmacokinetics of berberine and salvianolic acid B in single herb and traditional herbal medicine

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Traditional herbal medicines are consisted with several herbs and they have been interested in the field of new drug development due to high effectiveness and low toxicity. Owing to the chemical complexity, the specific and advanced analytical method will be necessary for the quality control and pharmacokinetic study of the herbal medicines. Wen-Pi-Tang-Hab-Wu-Ling-San (WHW), one of modified traditional herbal medicine, was developed and used for inhibition of the renal fibrosis in kidney cells. Coptidis Rhizoma and Salviae Miltiorrhizae Radix are the key ingredients of WHW. Berberine (BB) and salvianolic acid B (SB) are the representative components of both herbs, respectively. We compared the pharmacokinetic parameters of two representative components in single herb and WHW. For pharmacokinetic study of BB and SB, the relevant amount of Coptidis Rhizoma, Salviae Miltiorrhizae Radix and WHW was administered orally to rats. Blood samples were collected and analyzed by HPLC. HPLC analysis was performed by Shimadzu LC-10AD system with a diode array detector at 250 nm using C 18 column. Gradient program for mobile phases of 5% methanol containing 1% acetic acid and 95% methanol was used for the separation. In the validation of the method, the accuracy of intra- and inter-day was 99.6% ~ 104.0% and 98% ~ 102.4% for BB, and 101.1% ~ 103.0% and 93.0% ~ 105.8% for SB, respectively. The intra- and inter-day precisions of BB were below 2.93% and 4.98%, and those of SB were below 4.17% and 6.54%, respectively. For the animals received WHW in pharmacokinetic study, AUC was increased, while Tmax and T1/2 was decreased comparing with the animals received pure BB. However, for the WHW group, Cmax, Tmax, AUC and T1/2 were increased comparing with the animals received pure SB.

PI53 Anti-inflammatory effects of triterpene saponins from *Kalopanax pictus*

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Phytochemical study of the stem bark of *Kalopanax pictus* Nakai resulted in the isolation of 23 oleanane-type triterpenes and saponins, including eight new (**1 – 5** and **16 – 18**), and 15 known compounds (**6 – 15** and **19 – 23**). Their structures were identified by spectroscopic and chromatographic data. For the quantitation of saponins, the HPLC-ELSD system was used. The HPLC analysis was achieved on an Optimapak C_{18} column (4.6 mm x 250 mm, 5 μm, RStech Corp, Korea). The mobile phase consisted of solvent A (water) and solvent B (acetonitrile) was used in gradient elution: 0 min, 30% B; 15 min, 50% B; 20 min, 100% B and held at 100% for 10 min at the flow rate of 0.50 mL/min. The chromatograms were monitored at the following ELSD conditions: temperature 65°C, gas pressure at 2.0 bar. The results showed that the content of kalopanaxsaponin B, kalopanaxsaponin C and sieboldianoside A were in the range of 0.61~27.77 mg/g, 0.99~26.83 mg/g and 1.56~16.75 mg/g in KC samples, respectively. Seventeen of the compounds (**1 – 5**, **7 – 12**, **14**, **16**, **17**, **20**, **21**, and **23**) significantly inhibited TNFα-induced NF-*k*B transcriptional activity in HepG2, with IC_{50} values ranging of 0.6 – 16.4 μM. Compounds **9**, **11**, **12**, **14**, **16 – 18**, **20**, **21**, and **23** upregulated PPARs transcriptional activity in HepG2, with EC_{50} values of 0.2 – 15.5 mM. Compounds **14**, **15**, **19**, and **21** showed significant PPAR*a* transactivational activity, with EC_{50} values of 17.3, 8.0, 7.8, and 10.3 mM, respectively. Compounds **14**, **17**, **19**, and **20 – 22** exhibited PPARg dose-dependent transactivational activity, with EC_{50} values of 16.3, 14.7, 15.5, 14.8, 10.9, and 17.1 mM, whereas compounds **14** and **21** significantly upregulated PPARβ*(d)* transcriptional activity, with EC_{50} values of 17.7 and 15.7 mM, respectively. These results provide a scientific support for the use of the stem bark of *K. pictus* Nakaiand warrant further studies to develop new agents for the prevention and treatment of the inflammatory and metabolic diseases.

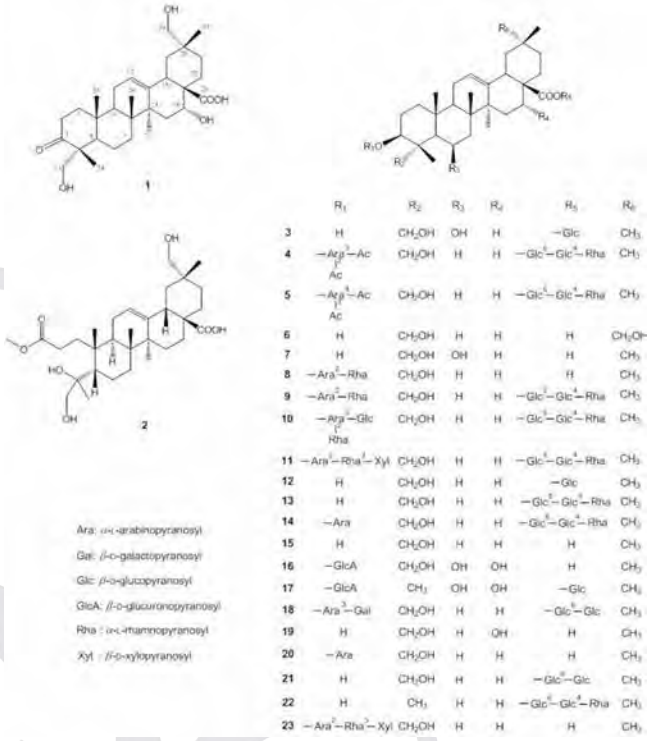

Fig. 1: Structures of isolated compounds

PI54 New secondary metabolites from *Gentiana* spec.

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The genus *Gentiana* (Gentianaceae) consists of many species with interesting phytochemical properties which have been widely used in traditional folk medicine [1]. Some members of *Gentiana* are used as folk remedies in Anatolia for their antipyretic, stomachic, stimulant of appetite, hepatoprotective, antidepressant and antidiabetic activities [2 – 5]. Iridoids, secoiridoids, *C*-glycosylflavones and xanthones are considered as the most promising groups of compounds responsible for the pharmacological activities of *Gentiana* species [1]. In a continuation of the phytochemical investigations on the genus, we have isolated one new flavonoid *C*-glucoside (**1**) along with 4 known compounds (**2 – 5**) from *Gentiana pyrenaica* and one new secoiridoid glucoside (**6**) and a known xanthone (**7**) from *Gentiana verna* subsp. *pontica*. The compounds were characterized by various chromatographic methods and the structures of the isolates were elucidated by means of spectroscopic (^{1}H-NMR, ^{13}C-NMR, 2D-NMR and Mass spectr.) evidence.

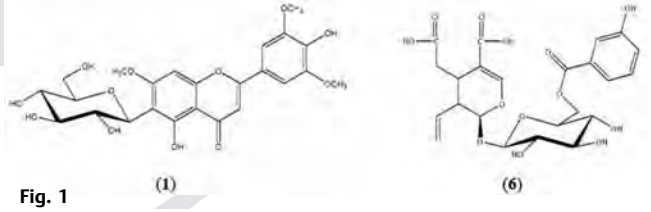

Fig. 1

Acknowledgement: This work was supported by Hacettepe University Research Foundation (Project No. 0601301004). **References:** [1] Jensen S.R., Schripsema J. (2002) *GENTIANACEA-Systematics and Natural History*, Struwe, L., Albert, V. Eds., Cambridge University Press. [2] Baytop, T. (1999) Therapy with Medicinal Plants in Turkey (Past and Present), 2nd edn. Nobel Publication, İstanbul. [3] Ersöz, T., et al. (1991) Fitoterapia 62: 184 – 185. [4] Sezik, E., et al. (2005) Life Sci. 76: 1223 – 1238. [5] Deliorman, O.D., et al. (2003) Life Sci. 72: 2273 – 2283.

PI55 *Allium* sulphur chemistry and traditional use of wild onions along the silk road

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The genus *Allium* L. (onions) contains more than 800 species, which were divided in several subgenera; Many of them have been used as phyto-pharmaceutics, seasonings, and vegetables. Most prominent are common onion (*A. cepa* L.) and garlic (*A. sativum* L.). The health benefits of *Allium* vegetables are mainly related to sulphur containing compounds as well as saponins. The species-rich genus *Allium* has a main centre of distribution reaching from Southwest Asia to the high mountains of Central Asia, were several wild species are used by the local population, as one can be concluded from casual remarks in some floras. Cysteine sulphoxides of these plants are believed to be mainly responsible for these health benefits. These compounds are converted to thiosulphinates like allicin by the enzyme alliinase, when plant material is disrupted. In this investigation, *Allium* samples mostly belonging to the subgenera *Allium*, *Melanocrommyum* and *Reticulatobulbosa* were chemically analysed. Amino acids as well methiin, alliin, isoalliin, homoisoalliin, propiin, marasmin, a pyrrole cysteine sulphoxide and cysteine pyridinyl N-O were determined by HPLC-MS/MS (Figure). As the only cysteine sulphoxide, marasmin contains two sulphur atoms. Comparable to all cyteine sulphoxides, the pyridinyl N-O is prone to allinase digestion. Most investigated species belonged to the subgenus *Melanocrommyum*. Usually methiin occurs in significant concentrations. *Allium* species of the subgenera *Allium*, *Rhizirideum* and *Cepa* did show higher concentrations of alliin, isoalliin and propiin. Substances of highest interest were cysteine derivatives with alkenyl or heteroaromatic residues. In total, several of the investigated species showed relative large amounts of cysteine sulphoxides (higher than 0.25%). Many of these species do show a traditional use.

PI56 On a search of biologically active compounds in bryophytes: Chemical composition and major secondary metabolites

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Bryophytes can be found in any location around the world. They are the second largest group in Plant kingdom, and they are divided in 3 groups-*Musci*, *Marchantiophyta* and *Anthocerotophyta.* It is assumed that bryophyte extracts have antimicrobial, antifungal and antitumor activity. Although there have been some studies in this area, it is important to take in account that bryophyte chemical composition may differ between species, growth environment and geographical localization. Based on this assumption bryophytes common for Northern Europe were analyzed. Twenty bryophyte species found in Latvia were analyzed. We have studied bryophyte basic chemical composition using elemental composition analysis, FTIR, ^{13}C NMR spectra and Py/GC/MS. Basic chemical composition analysis reveal nearly total absence of lignin and lignin like structures. To study secondary metabolites sequential extraction approach was used and the extraction efficiency was studied, including microwave extraction and others. In the extracts total characteristics such as dry residue, total phenolics, sugars, antioxidant activity and other were determined. Lipophilic extracts (obtained after extraction with chloroform) were analyzed using GC/MS. There have been found more than 60 different compounds. Bryophyte sample analysis using Py/GC/MS showed that the dominant compounds in lipophilic fraction of bryophytes were: phenol and 4-ethenylphenol. Results have shown that studied bryophytes contain some substances with aromatic ring, but none of the found bryophytes showed any prove of lignin content. The studied extracts of bryophytes demonstrate antimicrobial activity as demonstrated on example of *E. coli, B. cereus, P. aeruginosa, S. aureus, S. epidermidis, M. luteus, B. subtilis* as well as cytotoxic activity in respect to rat glioma, human epiderm carcinoma, human lung carcinoma and mouse melanoma cell line.

PI57 Triterpene saponins from *Chenopodium bonus-henricus* roots

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Chenopodium bonus-henricus L. has been recognized by Bulgarian legislation as a medicinal plant. Infusions of the roots have been used for treatment of bronchitis, laryngitis, reumatism, gout, constipation, dermatitis, eczema. The decoct of the roots of Good King Henry (also known as "chuven") is used for production of “tahin” and “white halva”. The present study investigated the hepatoprotective activity of MeOH extract from the roots of *C. bonus-henricus*, employing *in vitro* and *in vivo* hepatotoxicity models based on carbon tetrachloride (CCl_4)-induced liver damage in male Wistar rats. *In vitro* experiments were carried out in primary isolated rat hepatocytes. Cells pre-incubation with the extract significantly ameliorated in a concentration-dependent manner, the CCl_4-induced hepatic damage. Along with decreased malondialdehyde quantity and increased level of reduced glutathione (GSH), seven days pre-treatment of rats with the MeOH extract also prevented the CCl_4-caused oxidative damage by increasing antioxidant enzyme activities (catalase, superoxide dismutase, GSH-s-transferase, GSH peroxidase, GSH reductase). An extensive chromatographic procedure of the MeOH extract led to the isolation of one new as well as three known triterpene saponins. The new compound was identified as 3-O-β-*D*-glucopyranosyl-phytolaccagenin-28-α-*L*-arabinopyranosyl ester (**1**) on the basis of 1D, 2D-NMR techniques, (COSY, HMBC, and HSQC), MS analysis and chemical methods. The known compounds were identified as 3-O-β-glucuronopyranosyl-bayogenin-28-O-β-glucopyranosyl ester (**2**), 3-O-β-glucopyranosyl-bayogenin-28-α-*L*-arabinopyranosyl ester (**3**), 3-O-β-glucuronopyranosyl-medicagenic acid-28-β-xylopyranosyl(1→4)-α-rhamnopyranosyl(1→2)-α-arabinopyranosyl ester (**4**). Further studies are in progress to investigatethe hepatoprotective activityof the isolated saponins. **Acknowledgement:** *This study was supported by Medical Council at the Medical University of Sofia (Project 11/2013)*

PI58 VEP1- encoded enone 1,4 – reductases from Brassicaceae: cloning, expression, molecular phylogeny and modelling

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The mustard family (Brassicaceae) supplies the world with many crop species and medicinal plants. In the Brassicaceae only few genera contain cardenolides (*Syrenia, Draba, Erysimum* incl. *Cheiranthus*) whereas all of them contain glucosinolates. One may ask whether cardenolides originated independently several times in the Brassicaceae or whether they were lost during evolution in most of the genera. In order to shed light on the conservation and/or the multiple evolution of genes coding for cardenolide-biosynthetic enzymes, we isolated cDNA encoding progesterone 5β-reductases (P5βR) from several Brassicaceae species (*Armoracia rusticana, Aethionema grandiflora, Barbarea vulgaris,* various *Brassica* species*, Cakile maritima, Cardamine pratensis, Draba aizoides, Lunaria annua, Lepidium sativum, Matthiola tricuspidata, Nasturtium officinale, Raphanus sativus* and *Sisymbrium officinale).* P5βRs, are members of the short chain dehydrogenases/reductases (SDR) superfamily of proteins and are supposed to be involved in the biosynthesis of 5β-cardenolides. These enzymes have been characterized as substrate-promiscuous enone 1,4-reductases and are encoded by Vein Patterning 1 (*VEP1*) genes, which occur in a wide range of plant species independent of their ability to produce cardenolides or not. The nucleotide and deduced amino acid sequences of the new P5βRs were aligned with known and putative *VEP1*-encoded P5βRs and a cladistic tree was constructed which fitted well to the accepted Brassicaceae phylogenetic relationship, indicating a common ancestor for all Brassicaceae P5βRs. The new P5βR cDNAs were over-expressed in *E. coli* and the respective P5βR proteins tested for their catalytic function. Their kinetic constants were determined using progesterone and small enones as substrates. Molecular modelling was applied to elucidate structural and functional relationship between *VEP1*-encoded P5βRs.

PI59 New compounds from *Metaxya rostrata*

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Metaxya rostrata C. Presl (Metaxyaceae) is a tree fern and its rhizome is used as traditional herbal remedy against intestinal diseases in Costa Rica. Until now, very unusual methylene-cylcopropane glucosides, betulinic acid, polyphenols, glycosides of phenolic acids and common sterols are known from the rhizome of this plant [1]. In recent investigations, the very potent cytotoxic substance 2-deprenyl-rheediaxanthone B, which causes active cell death by a mechanism similar to mitotic catastrophe, was isolated [2]. In continuation of these studies, the aqueous and the ethylacetate extract of the rootlets were further analyzed and fractionated by partition and different chromatographic methods such as vacuum liquid chromatography on silica, gel permeation chromatography on Sephadex® LH20 and solid phase extraction on RP-18 as stationary phases. Besides the known compounds squalen and fern-9(11)-en [3], four new natural compounds were isolated. Their structures were elucidated on the basis of detailed spectroscopic data analysis (NMR, MS) as (4'E)-1-O-(β-glucopyranosyl)-*N*-(2'-hydroxytetracosanoyl)-4,8-sphingadienine (d18:2/h24:0-GlcCer) (**1**), to our knowledge the first glucoceramide identified in a fern, (2E)-2-(hydroxy-hexyliden)cyclopropyl-1®3-diglucoside (**2**), (6E)-6[2-(ß-glucopyranosyloxy)cyclopropyliden]-hexanoic acid methylester (**3**) and 2-deprenyl-7-hydroxy-rheediaxanthone B (**4**).

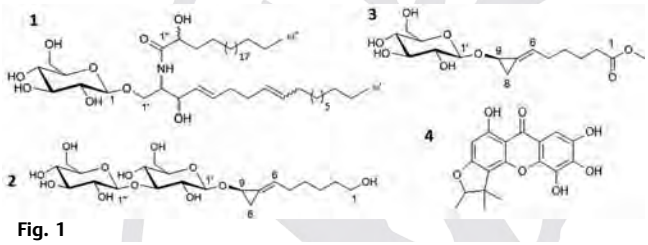

Fig. 1

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PI60 Biological activities and chemical constituents of *Ferula anatolica* Boiss.

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Genus *Ferula* has about 185 species and widely distributed through out Central Asia, China, Afghanistan, India, Syria, Palestine, Israel, North Africa, North America, South-West and Central Europe. Currently, 23 species and 4 subspecies of Ferula are known from Turkey and 12 of them are endemic. Chemical composition of 70 *Ferula* species were investigated by phytochemical studies so far; glucuronic acid, galactose, arabinose, rhamnose, sulphur containing compounds, coumarins, sesquiterpenes, sesquiterpene coumarins, sesquiterpene lactones and daucane esters (Miski, 1985, Ahmed, 1999) were isolated. In this study, *Ferula anatolica* Boiss. which is endemic species growing in Turkey, have been investigated for its chemical constituents and cytotoxic and antioxidant activity. The air dried and powdered underground parts of plant extracted with soxhlet apparatus with petroleum ether, dichloromethane and methanol. The petrolerum ether extract of *F. anatolica* was used for isolation studies. By means of a serial chromatographic studies performed on the extract five sesquiterpene derivatives compounds were isolated. The structures of compounds were elucidated using spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, IR, Mass spectr.) In study of Antioxidant activity, methanol and dichloromethane extracts, DPPH and ABTS radicals scavenging, reducing Fe(III) to Fe(II) and inhibiting of lipit peroxidation were determined to have antioxidant activity. In MTT and LDH tests that we have done a comparative cytotoxicity studies, the MTT test, cytotoxic activity was observed, LDH test, only the dichloromethane extract showed very low activity. **References:** [1] Ahmed A.A, Sesquiterpene coumarins and Sesquiterpenes from *Ferula sinaica*, Phytochemistry, 50, 109 – 12, (1999). [2] Miski, M. Mabry, T.J., Daucane Esters From *Ferula communis* subsp. *communis*, Phytochemistry, 24, 1735 – 41, (1985).

PI61 A bioassay-guided phytochemical study of extracts from *Vernonia crotonoides* active against multi-resistant hospital bacteria

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In our continuous searching for new natural products active against resistant pathogens, the vegetal species *Vernonia crotonoides,* colected in Jurubatiba shoal, Rio de Janeiro, was selected for this study. The main goal of this investigation was to evaluate the antibacterial activity of extracts from *V. crotonoides* leaves and, to identify the compounds responsible for the activity. The crude ethanol extract was submited to a liquid-liquid partition furnishing the *n*-hexanes, dichloromethane (DCL), ethyl acetate, buthanol and aqueous fractions. For the antibacterial activity there were applied the agar dilution and the disk diffusion techniques, both standardized by the National Committee for Clinical Laboratory Standards. The ethanolic extract and the fractions were tested at 512, 256, 128 and 64 μg/mL against 16 oxacillin-resistant *Staphylococcus aureus* strains from nosocomial sources and 12 ATCC strains, including gram-positive and gram-negative bacteria. The results showed that at 512 μg/mL the DCL fraction inhibited 15 *S. aureus* oxacillin-resistant strains and 11 ATCC strains. At 256 μg/mL and 128 μg/mL this fraction inhibited 4 and 2 strains, respectively. The other fractions did not present any relevant activity. By the disk diffusion method the DCL promoted great inhibition zones that varied from 12 mm to 23 mm against *S. aureus* oxacillin-resistant strains. The antibiotics vancomicine and oxacillin were used as control (d = 13 mm). Based on the promissing results, the DCL was fractioned by silica gel column chromatography producing about 500 fractions that were assembled based on their similarity profile by TLC. The fractions were analyzed by GC-MS, which showed the presence of sesquiterpenes. The pure substances will be analyzed by the NMR technique and also tested for their antibacterial activity. The results presented demonstrate that *V. crotonoides,* for the first time studied by this propose, can represent a promising new source of antibacterial agents.

PI62 Microwave-assisted extraction of metabolites from *Humiria balsamifera* leaves: a rapid and efficient methodology for antioxidant constituents obtainment

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The microwave-assisted extraction (MAE) presents some advantages compared to the conventional method like lower extraction time and solvent consumption, besides being more selective. This study aimed to combine important parameters for MAE by using *Humiria balsamifera* leaves, from Restinga Jurubatiba, in comparison to the maceration method in terms of its flavonoid contents, the antioxidant activity and the crude yield. For microwave extraction (MO), a 2^3 factorial design was performed, combining three factors: solvent proportion (ethanol: water 1:1)/mass, temperature and stirring in 30 min. Concomitantly, a maceration extraction was also done during 7 days with the same solvent. The solutions were concentrated, the yields calculated and then analyzed by HPLC-DAD for the total flavonoids dosage. The antioxidant activity was performed by the DPPH method (2,2-diphenyl-1-picryl-hidrazil), in which the standardized EGb 761® extract of *Ginkgo biloba* was used as standard. The best yield (18.83%) was observed under the following conditions: higher proportion (1:20), higher rotation (1000 rpm) and higher temperature (55 °C), being significantly greater when compared to the maceration (5.13%). The calibration curve allowed the quantification of flavonoids with the UV spectra equivalent to rutine (λmáx 202, 255, 354 nm). To the antioxidant activity, the results were expressed as EC_{50} values, where the sample in the same conditions showed the lowest EC_{50} (33.06 ± 0.73), which is also in accordance with the result ob-

tained for total flavonoids (7.94 EqR%). All samples, including that prepared by the conventional method exhibited best antioxidant activity compared to the standard (EC_{50}= 45.07 ± 0.15), already recognized for showing outstanding antioxidant activity. MAE was more efficient, showing higher yields in lower time in comparison to the maceration, thus achieving favorable conditions. The model was satisfactorily explained by the experimental design.

PI63 Chemical constituents from the fermented broths of *Coprinellus radians*

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In our preliminary screening, the ethyl acetate extracts of the fermented broths of *Coprinellus radians* #YMJ1168 were found to exhibit moderate growth inhibitory activity against A549 lung cancer cell line with a GI_{50} value of 55.1 μg/mL. Thus, a broths of this fungus were carried out, which resulted in the isolation and identification of five compounds **1**-**5**. Their structures were elucidated to be coprinol (**1**), guanacastanes P (**2**), J (**3**), E (**4**) and N (**5**) based on spectroscopic analyses. Among these, **1** and **2** were new, and **3** was isolated from natural resources for the first time. The growth inhibitory activities of **1**, **3**, **4**, and **5** against A549 were evaluated, and only **2** exhibited moderate activity with a GI_{50} value of 18.2 μM. Moreover, by adding suberoylanilide hydroxamic acid (SAHA, 10 μM), the histone deacetylase inhibitor, in the culture medium of this fungal strain, an unexpected component was found, which showed a molecular ion $[M - H]^-$ at *m/z* 507 by LC/Q-TOFMS analysis. However, the structure of this component remained to be further investigated.

PI64 Trichophenols A-D, four novel phenolic ethers from the fermented broths of *Trichobotrys effusa* YMJ1179

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Four novel phenolic ethers, namely trichophenols A-D (**1**-**4**), were isolated from the ethyl acetate extracts of the fermented broths of *Trichobotrys effusa* YMJ1179. The structures of **1**-**4** were elucidated based on spectroscopic data analyses. The absolute configuration of **1** was determined by modified Mosher's method. Compounds **1**-**4** exhibited moderate growth inhibitory activity against A549 lung cancer line with GI_{50} values of 25.6, 19.3, 16.2 and 24.3 μM, respectively. Moreover, **1** exhibited moderate growth inhibitory activity against TOV-21G-Tx paclitaxel-resistant ovarian cancer cell line with a GI_{50} value of 21.3 μM.

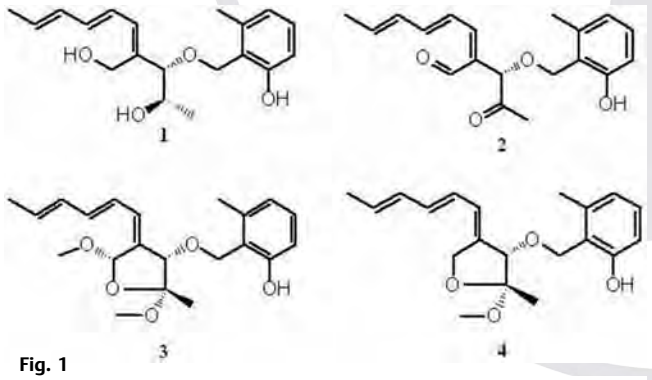

Fig. 1

PI65 Investigation of flavonoids from *Siparuna sarmentosa* Perkins

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Siparuna sarmentosa Perkins (Siparunaceae), known as 'Limão-bravo' is an herbaceous plant of an important medicinal genus from Brazilian Amazon. There are reports of medicinal uses of several species of *Siparuna* by indigenous and local communities. Literature reports the isolation of flavonoids, alkaloids and terpenoids from this genus but there are no reports on the chemistry of the species *Siparuna sarmentosa* (Ss). Previous work on *S. guianensis* by HPLC-DAD-ESI-MS/MS [Negri,2012] reports the presence of *C*-glycosylated flavonoids, never before reported for *Siparuna.* In this way, this study aims to evaluate the chemical profile of Ss by TLC, HPLC-DAD and HR-ESI-MS and to isolate the flavonoid derivatives by CCC in order to confirm the *C*-glycosyl pattern. Leaves of Ss, collected in the National Park Adolpho Ducke (Manaus, Brazil) were extracted with methanol and the crude extract obtained (SsEM) was subjected to clean-up by SPE. The clean-up performed by SPE was carried out in reversed phase (RP18) and the yielded fractions were analyzed by HR-ESI-MS. The error for each suggested molecular formula was calculated in ppm. The presence of lucenin-2 (1.5 ppm) and vicenin-2 (0.3 ppm), vitexin (4.6 ppm), quercitrin (2.9 ppm) and vanillic acid (0.4 ppm) [Negri,2012] is suggested. In order to obtain an enriched flavonoid fraction for further isolation of the proposed *C*-glycosyl flavonoids, high-speed countercurrent chromatography (HSCCC) of SsEM was performed with the biphasic solvent system hexane-ethyl acetate-methanol-water 3:6:6:5, in reversed phase mode, affording the flavonoid fraction SsFF. In this process a flavonoid aglycone and a terpenoid derivative were recovered from the organic stationary phase of the HSCCC fractionation. Their structure is under elucidation. **Reference:** [1] Negri, G., Santi, D., Tabach, R. *Braz. J. Pharmacognosy*, 22(5), 1024 – 1034, 2012.

PI66 Secondary metabolites from the stem of *Neolitsea daibuensis* and their anti-inflammatory activity

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Bioassay-guided fractionation of active ethyl acetate-soluble layer from the setm of *Neolitsea daibuensis* Kamikoti led to the isolation of 28 compounds, including 13 new sesquiterpenoids: elemanodaibulactones A-C, daibudilactones A-E, daibulactones D-G, and daibuguaianin. The structures of these isolates were elucidated by spectral analysis and single-crystal X-ray diffraction. Daibudilactone A, daibudilactone B, daibudilactone C, daibudilactone D, elemanodaibulactone A, and daibudilactone E exhibited potent anti-inflammatory activity using an inducible enitric oxide synthase (iNOS) assay, with IC_{50} values of 0.44, 2.73, 1.48, 13.78, 6.70, and 4.73 μM, respectively.

PI67 Sequestration and biotransformation of lignans from *Aristolochia giberti* by *Battus polydamas* larvae (Papilonidae: Troidini)

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A previous chemical study on *Aristolochia giberti* led to the isolation of fourteen compounds, including three lignans [1]. Dibenzylbutyrolactone lignans exhibit insect feeding deterrent activity that is strong enough to mediate plant-insect interactions [2]. Lignans with methoxy and/or methylenedioxy substituents are more actives than those with hydroxyl and glucosyl groups [2]. In addition to deterring appetite on insects, lignans can kill or hindrance their life cycles [3]. In general, lepidopteran larvae convert lignoids into its aromatic ring *O*-demethylated, hydroxylated, and glycosylated derivatives [4]. In this study, *Battus polydamas* larvae were fed at laboratory with leaves of *A. giberti*, and the organic extracts of leaves (LE), feaces (FE), and larvae (LVE) were prepared by maceration. From these extracts, a know lignan, kusunokinin (**1**), was isolated. Whereas, from the FE two new dibenzylbutane lignans, 2-(4',5'-methylenedioxybenzyl)-3-(4'',5''-dimethoxybenzyl)-4-*O*-β-glucopyranyl-butan-1-oic acid (**2**) and 2,3-[4',5':4'',5''-bis(methylenedioxybenzyl)]-4-*O*-β-glucopyranyl-butan-1-oic acid (**3**), were isolated and their structures determined by spectroscopic analyses. Compound **4** was

identified in LE and FE by HPLC-PDA as cubebin. Compounds **1** and **5** were identified in LVE by CG-MS analyses. It is known that kusunokinin (**1**) causes high mortality in larvae of soybean caterpillars [3], whereas cubebin (**4**) and hinoquinin (**5**) are insect feeding deterrents [2]. Therefore, it is reasonable to propose that **2** could be a product from **1**, whereas **3** be a biotransformed product from the **4** or **5**. Hence, *B. polydamas* may have their own strategy to overcome chemical barriers imposed by *A. giberti*. **References:** [1] Lopes, L. M. X. et al. (2009) J. Braz. Chem. Soc. 2: 19 – 108. [2] Harmatha, J., Nawrot, J. (2002) Entomol. Exp. Appl. 104: 51 – 60. [3] Messiano, G. B. et al. (2008) J. Agric. Food Chem. 56: 2655 – 2659. [4] Ramos, C. S. et al. (2008) Phytochemistry 69: 2157 – 2161.

PI68 Studies on the phenolic content of Romanian Feteasc neagr wines

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The phenolic compounds in plants and the related foods inhibit the oxidation of lipoproteins with low density and reduce the plaquetary aggregation in blood vessels. Red wine is an excellent source of phenolic compounds, registering between 1000 – 4000 mg/L flavonoids with diverse biological effects [1]. Phenolic compounds also can contribute to the organoleptic characteristics of red wines. Therefore, the study of the wine-making technology influence on the phenolic content of 8 Feteasc neagr wine samples was considered important. The analyses were processed using high performance liquid chromatography coupled with a diode array detector. Feteasc neagr grape variety harvested in 2009 and 2010 from Uricani vineyard was used, as follows: V1 and V2 wine samples were processed according to classical red wine-making procedure; V3 and V4 were obtained by applying thermo-maceration on the grape marc; V5 and V6 resulted through thermo-maceration of grape marc in roto-tanks; V7 and V8 were blanc de noir variants. After fermentation, the wines were stabilised, conditioned and bottled. All samples were analyzed on a Shimadzu Prominence 20 series HPLC system. Phenolic compounds were separated on a series of two Chromolith Performance RP-18e chromatographic columns, from Merck (2). Analyses were performed in triplicate and the data was presented as mean ± - standard deviation (SD). The quantity of phenolic compounds is influenced by the agricultural year as well as the processing technology (Table 1). The 2009 V3 wine sample obtained through thermomaceration registers a significant increase of concentration in caffeic, ferullic, *p*-hydroxybenzoic, gallic acids and a decrease of the syringic and gentisic acids.

Tab. 1: Concentration of polyphenolic constituents in mg/L in wine samples processed in 2009 (VI, V3, V5, V7) and in 2010 (V2, V4, V6, V8)

No.	Wine sample	proto-catehic acid	*p*-hydroxy-benzoic acid	vanillic acid	gallic acid	syringic acid	gentisic acid	caffeic acid	chlorogenic acid	*p*-couma-ric acid	ferulic acid
1	FN V1	3.27	0.28	3.20	16.43	4.58	1.02	0.14	2.72	2.90	0.04
2	FN V2	3.16	0.50	3.82	22.73	5.36	1.04	7.42	2.39	4.24	0.64
3	FN V3	3.76	5.03	5.89	25.90	3.50	0.61	13.51	5.00	6.47	1.23
4	FN V4	4.25	022	3.79	29.01	5.33	0.74	7.70	2.39	5.30	0.91
5	FN V5	3.11	0.34	3.69	24.40	6.72	0.65	17.79	2.40	3.70	0.08
6	FN V6	5.05	8.67	3.09	18.93	4.28	2.12	6.44	2.40	4.09	0.05
7	FN V7	1.38	0.41	8.79	10.08	1.97	0.50	8.90	2.32	3.57	0.04
8	FN V8	1.17	1.05	9.79	12.39	3.06	1.38	8.23	1.98	3.91	0.03

References: [1] Arnous, A., Makris, D. P., & Kefalas, P. (2001), *Journal of Agricultural and Food Chemistry*, 49, pp.5736 – 5742 [2] Castellari, M., Sartini, E., Fabiani, A., Arfelli, G., Amati, A., *Journal Chromatography A*, (2002), 973, pp.221 – 227

PI69 Isolation of Actinomycin D from marine fauna associated bacteria

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Two antibiotic producing bacterial strains, one (SS23) from marine sediments and another (CN3) from the venom duct of the marine cone snail *Conus monile* have been isolated. On the basis of morphology, phenotypic and genotypic characteristics, the bacteria have been shown to belong to the genus *Streptomyces*. Purification of bioactive principles was achieved by solvent extraction, fractionation by silica gel chromatography and finally by high performance reverse phase liquid chromatography using a C18 column. Antimicrobial activity studies were carried out to identify bioactive fractions. Of the HPLC fractions, one fraction a MIC of 1 mg/ml against *B. subtilis*, *E. coli* and methicillin resistant *S. aureus* and a MIC of 50 mg/ml against *C. albicans*. Mass spectrometric analysis showed that this HPLC fraction had a single compound that had a mass of 1255 Da. Further activity studies showed that this molecule exhibited potent activity against *M. tuberculosis* (MIC of 10 mg/ml). The compound also showed potent cytotoxic activity against breast (MCF-7), melanoma (A375), prostate (DU145) and lung (A549) cell lines at IC concentration values of 20 mg/ml. Molecular structural analysis of this compound using a combination of tandem mass spectrometry and multidimensional NMR spectroscopy (Figure.1) has shown that this compound is Actinomycin D. Sequencing of the genomic DNA of SS23 and CN3 are currently in progress to establish the identity of the species.

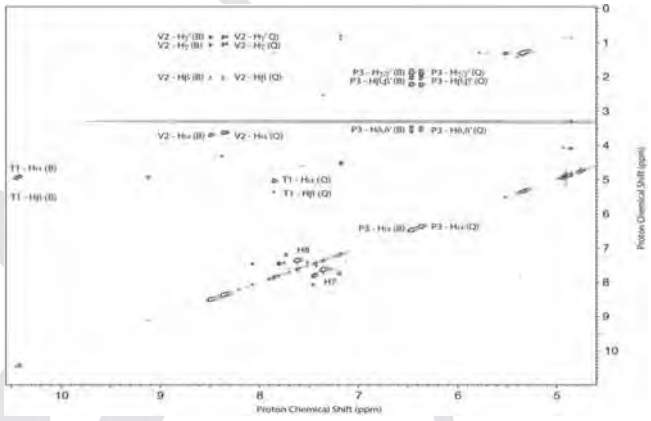

Fig. 1: Finger print region (H^N-Hα) of the two dimensional ^{1}H-^{1}H TOCSY NMR spectrum. Residues specific assignments are indicated in the spectrum.

PI70 Screening of specific photoprotective compounds in Ulvophyceae (Chlorophyta) from the Southeastern Brazilian coastline

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Ultraviolet radiation exerts its deleterious effects on terrestrial and aquatic ecosystems. To avoid damage, organisms developed defense mechanisms such as the synthesis and accumulation of mycosporine-like amino acids (MAAs). MAAs are water-soluble, small molecules (see figure below) having an absorption maxima of 309 – 362 nm, synthesized only by fungi, algae and bacteria. Besides having photoprotective function, they are believed to have other biological roles, such as contributions to osmotic regulation, reproduction and chemical signaling[1].

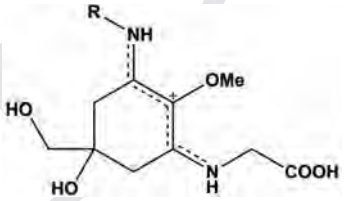

Fig. 1: General structure of a mycosporine-like amino acid.

In order to obtain more information about Brazilian seaweed, seven samples of Ulvophyceae (Chlorophyta) were collected at the south coast of Espírito Santo State (Southeast Region of Brazil): *Caulerpa racemosa* var. *occidentalis, Caulerpa cupressoides* var. *lycopodium*, *Chaetomorpha antennina*, *Codium decorticatum*, *Udotea flabellum* and *Halimeda cuneata* (two samples collected at different sites). It was possible to separate and identify some MAAs by making use of HPLC-MS. Shinorine and porphyra-334 seem to be ubiquitous among these algae; indeed, these compounds are described as the most common MAAs found in nature.

The two samples of *H. cuneata* exhibit different patterns of MAAs, despite their collection sites being quite close to each other; however, the peak distinguishing one sample from the other has not yet been identified. Among the studied algae, *C. racemosa* var. *occidentalis* seems to be the most interesting one, given its higher diversity of peaks: with absorption maxima ranging from 319 nm to 343 nm (to say, both in UVA and UVB spectrum ranges), this alga extract may become an attractive alternative for new sunscreen formulations. It was not possible to accurately identify these peaks until now, but more analyses are being carried out to identify these compounds. **Reference:** [1] *Carreto JI, Carignan MO.* Mycosporine-like amino acids: Relevant secondary metabolites. Chemical and ecological aspects. Mar. Drugs 2011; 9: 387.

PI71 Flavonoids from *Moraea sisyrinchium* (L.) Ker Gawl. (Iridaceae) in Egypt

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The first phytochemical investigation of *Moraea sisyrinchium (L.)* Ker Gawl. revealed the isolation and elucidation of seven flavonoids; apigenin, apigenin 7-O-β-glucopyranoside, luteolin 7-O-β-glucopyranoside, isovitexin, orientin, isoorientin and saponarin. Their structures were established on the basis of chemical and spectroscopic analysis and by comparison with the literature data. The chemotaxonomic significance was investigated.

PI72 Isolation & Structure Elucidation of Acylphloroglucinols from *Hypericum amblycalyx* Coustur. & Gand.

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Acylphloroglucinols are a common class of bioactive phenolic compounds and can be found in the familiy Hypericaceae. The large structural diversity results from different kinds of linked acyl-moieties as well as different numbers of prenyl- or geranyl-moieties resulting in mono-, bi- & tricyclic compounds. The aim of this project was to determine the acylphloroglucinol spectrum of *Hypericum amblycalyx* which is an endemic plant growing in the east of Crete. It is taxonomically classified in the sectio Coridium (section 19) [1]. By ^{1}H-NMR-guided fractionation, four acylphloroglucinol derivatives were isolated using silica- and RP18-flash chromatography and semi-prep HPLC, and their structures were confirmed by modern spectroscopy (1D- and 2D-NMR, ESI-MS): the two monocyclic derivatives 4-geranyl-2-(2'-methylbutyryl)-phloroglucinol and 4-geranyl-4-(2'-isobutyryl)-phloroglucinol, as well as two bicyclic compounds 1-[(2R*, 3S*)-3,5,7-trihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-8-yl]-2-methylbutan-1-one and 1-[(2R*, 3S*)-3,5,7-trihydroxy-2-methyl-2-(4-methylpent-3-enyl)chroman-8-yl]-2-methyl-propan-1-on. All identified acylphloroglucinols are also described for the previously investigated *H. empetrifolium* [2, 3], belonging to the same section, i. e. Coridium; these data give evidence to their taxonomic correlation. **References:** [1] Robson N. K. B., 2010. Studies in the genus *Hypericum* L. (Hypericaceae) 5 (2). Sections 17. Hirtella to 19. Coridium. Phytotaxa 2010; 4: 127 – 258; [2] Schmidt S., Jürgenliemk G., Skaltsa H., Heilmann J., 2012a. Phloroglucinol derivatives from *Hypericum empetrifolium* with antiproliferative activity on endothelial cells. Phytochemistry 77: 218 – 225; [3] Schmidt S., Jürgenliemk G., Skaltsa

PI73 Secondary metabolites from *Scutellaria velenovskyi* Rech. f.

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The aim of this study was to determine the chemical profile of *Scutellaria velenovskyi* (Lamiaceae), which is endemic to the Balkan Peninsula and Turkey [1]. It is taxonomically classified to the *S. albida* group. The plant material was collected from Karabük, Turkey. The methanol extract of the aerial parts was fractionated using RP_{18}-MPLC, flash chromatography and semi-prep RP_{18}-HPLC. Three iridoids, catalpol, albidoside, macfadienoside, four phenylethanoid glycosides, verbascoside, martynoside, leucoseptoside A, glucopyranosyl-(1 – 6)-martynoside, one phenolic derivative, *p-(E)*-coumaroyl glucopyranose and three flavonoids, scutellarein 7-*O*-β-glucuronopyranoside, hispidulin 7-*O*-β-glucuronopyranoside and eriodictyol were isolated and identified. The structures of the isolates were elucidated by spectroscopic methods including 1D- & 2D-NMR, UV-Vis. The chemical profile of this taxon is quite similar to that of previously investigated species belonging to the *S. albida* group, i. e. *S. albida* subsp. *albida* [2, 3], *S. albida* subsp. *colchica* [4] and *S. goulimii* [5]. **References:** [1] Bothmer, R. (1985) Nord. J. Bot. 5: 421 – 439. [2] Gousiadou, C. et al. (2007) Phytochemistry, 68: 1799 – 1804. [3] Gousiadou, C. et al. (2012) J. Enzyme. Inhib. Med. Chem. doi:10.3109/14756366. [4] Çalis, I. et al. (1993) Phytochemistry 32: 1213 – 1217. [5] Gousiadou, C. (2012) BSE. 43:139 – 141. **Acknowledgements:** This research has been co-financed by GSRT (grant 84889) and TUBITAK (SBAG-109S480).

PI74 Phenolic compounds in olives and olive oil from Algerian and Italian *Olea europaea* cultivars and their antioxidant activity

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Olive fruits (OF) and virgin olive oil (VOO) have nutritional and sensory characteristics that make it unique and a basic component of the Mediterranean diet.[1] The chemical composition of phenolic of OF and VOO is mainly affected by the variety, location, and environmental conditions.[2] Phenolic compounds of OF and VOO affect olive and oil shelf lifes since they retard oxidation and the sensorial properties: color, astringency, bitterness, and flavour.[2,3] and possess protective effects on human health, including cancer and cardiovascular diseases. In the present study, the identification and quantitation of the most representative phenolic compounds present in OF and VOO of two Algerian *(chamlal and sigoise)* and some Italian cultivars (*leccino cat, ogliarola bradano, maiatica pandolfo, frantoiana pandolfo, leccino pandolfo, frantoiana cat, coratina*) using HPLC-DAD based method is reported. HPLC-DAD HRMS was also used to confirm results obtained. Quantitative or semi-quantitative information on OF and VOO phenols is of great interest to find the compounds responsible of the olive oil benefits and to distinguish VOOs with different geographical origin.[2,3] Analyzed olive extracts and oils demonstrated significative differences ($p < 0.05$) in secondary metabolite identified, expecially concerning phenolic acids. The antioxidant activity was also measured using: 2,2-diphenyl-1-picrylhydrazyl, ferric reducing antioxidant power and β-carotene bleaching assays, [4,5] and relative antioxidant capacity index.[5] According to all tests, the highest hydroxytyrosol amount and antioxidant capacity were observed for the Italian cultivars *coratina* and *ogliarola*, and the Algerian cultivar *chamlal*. **References:** [1] Cioffi G. et al. *Food Chemistry* 2010, *121*, 105 – 111. [2] Keceli T., and Gordon, M. H. *J. Sci. Food Agric.* 2001, *81*, 1391 – 1396. [3] Esti, M. et al. *J. Agric. Food Chem.* 1998, *46*, 32 – 35 [4] Padula M.C. *Food Chemistry* 2012, in press [5] Russo, D. et al. Pharmacologyonline 1 (SPL. 1), 2012, pp. 84 – 93

PI75 Antidiabetic and cytotoxic activities of *Gleditsia triacanthos* L. leaves

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Gleditsia triacanthos L. is a deciduous tree belonging to family the Fabaceae. It possesses important biological activities as anti-mutagenic, anticancer, cytotoxic and treating rheumatoid arthritis (Miguel *et al.*, 2010). Eight flavone glycosides and two flavone aglycones named vicenin-I (I), vitexin (II), isovitexin (III), orientin (IV), isoorientin (V), luteolin-7-O-ß-glucopyranoside (VI), luteolin-7-O-galactopyranoside (VII), apigenin-7-O-ß-glucopyranoside (VIII), luteolin (IX) and Apigenin (X) were isolated from the aqueous ethanol extract of *Gleditsia triacanthos* L. leaves. The total ethanol extract of the dried powdered leaves of the plant as well as the successive extracts, petroleum ether, chloroform, ethyl acetate, and aqueous ethanol extracts were screened for their antidiabetic and cytotoxic activities. LD_{50} of the total ethanol extract was found to be 6.6 g/kg b.wt. Total ethanol and aqueous ethanol extracts at 100 mg/kg exhibited 68.72% and 62.95% potency respectively as compared with metformin (100% potency) in decreasing the blood glucose level after four weeks. The total ethanol extract showed a significant cytotoxic activity against breast (IC_{50}= 0.74 µg), larynex (IC_{50}= 0.67 µg), cervix (IC_{50}= 1.28 µg), liver (IC_{50}= 1.68 µg), and colon cell lines (IC_{50}= 2.62 µg) compared with cisplatin. Significant cytotoxic activities of compounds II, IV, VI and VIII against, breast, colon and liver cell lines were also proved **Reference:** [1] Miguel A., Cerqueira, Bartolomeu W.S. Souza, Joana T. Martins, José A. Teixeira, António A. Vicente (2010) Seed extracts of *Gleditsia triacanthos*: Functional properties evaluation and incorporation into galactomannan films *Food. Research. International.* 43, 2031 – 2038

PI76 Polyprenylated phloroglucinol derivatives from *Hypericum maculatum*

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Hypericum maculatum Cranz. is widely spread in all Bulgarian mountains and in many European countries. This species has been used in traditional medicine as an equivalent to the official drug Hyperici herba and has been given in several pharmacopoeias together with *H. perforatum* as a component of this drug [1,2]. Previous phytochemical studies of this plant have established the presence of naphthodianthrones hypericin and pseudohypericin, flavonoids kaemferol, quercetin, quercitrin, isoquercitrin, hyperoside, rutin, I3,II8-biapigenin and amentoflavone, xanthones norathiriol, maculatoxanthone, mangiferin, isomangiferin, kielcorin, phloroglucinol hyperforin as well as benzophenones annulatophenone, annulatophenonoside and acetylannulatophenonoside [3]. The extensive chromatographic procedure of the dichloromethane extract of the aerial parts of *H. maculatum* led to the isolation of five new (**1-5**) and five known (**6-10**) polyprenylated phloroglucinol derivatives as well as β-sitosterol. The new compounds were identified by means of spectral methods (MS, NMR, IR, UV). The cytotoxicity on SKW-3, BV-173 and K-562 tumor cell lines of these natural products were established using MTT test.

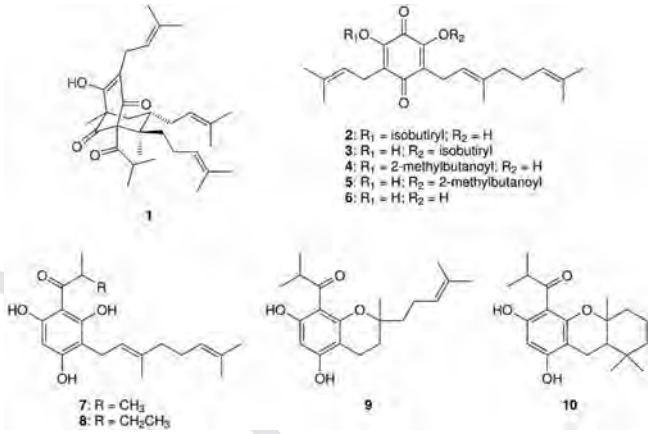

Fig. 1: Structures of compounds **1-10.**

Acknowledgements: This research was supported by a grant from the Medicinal Science Council at Medical University of Sofia (Project 52/2013). **References**: [1] Kitanov (2000) *Acta Pharm. (Zagreb)* **50**: 65 – 68. [2] Martonfi *et al.* (2006) *Biologia (Bratislava)* **61**: 473 – 478. [3] Zheleva-Dimitrova et al. (2012) *Nat. Prod. Res.* **26**: 1576 – 1583.

PI77 Anticonvulsant effects of kaurenoic acid isolated from the root bark of *Annona senegalensis*

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The herbal preparations of *Annona senegalensis* Pers. (Annonaceae) root bark are used in Nigerian ethnomedicine for the treatment of epilepsy and febrile seizures. The scientific evidence to this effect has been reported [1]. This study aims to identify and characterize the active constituent(s) responsible for the anticonvulsant effect. Bioactive-guided fractionation of the methanol-methylene chloride root bark extract (MME) of *A. senegalensis* using pentylenetetrazole (PTZ)-induced seizures in mice, afforded a potent anticonvulsant ethyl-acetate fraction (EF). Further fractionation of the EF yielded eight sub-fractions (F_1-F_8) which were tested for anticonvulsant activity. The sub fraction F_2 yielded whitish crystals that were purified to obtain *A. senegalensis* crystals, AS2. The AS2, which exhibited potent anticonvulsant effects, was characterized by 1D and 2D NMR spectroscopy, mass spectroscopy and X-ray crystallography. The AS2 was characterized as kaur-16-en-19-oic acid (KA), a diterpenoid phytoconstituent and exhibited the most potent anticonvulsant effect. The AS2 indicated an estimated LD_{50} of 3800 mg/kg. The results showed that the MME, EF and AS2 significantly ($P < 0.05$) and dose dependently delayed the onset of myoclonic spasms and tonic-clonic phases of seizures induced by PTZ and maximal electroshock seizure (MES). The anticonvulsant effects of the MME, EF and AS2 indicated the possible mediation of the anticonvulsant activity through central inhibitory mechanisms. Kaurenoic acid was identified as the anticonvulsant principle in the root bark extract of *A. senegalensis*.

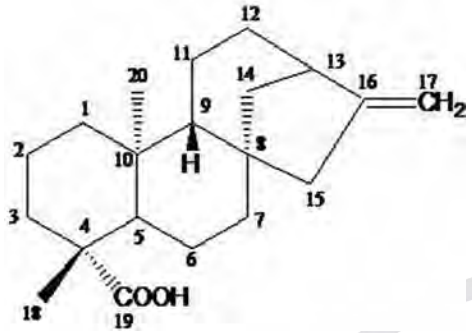

Fig. 1

Reference: [1] Okoye TC and Akah PA. (2010). Anticonvulsant and sedative effects of root bark extract and fractions of *Annona senegalensis*. Inventi Impact: Ethnopharmacology. 1(2): 100 – 103. **Acknowledgement:** The authors are grateful to Step-B Project of the Federal Government of Nigeria for the Innovators of Tomorrow (IOT) Award grant.

PI78 Immunomodulatory effect of *Landolphia owariensis* P. Beauv stringy seed pulp

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Landolphia owariensis P. Beauv, economically important for latex/rubber and folklore medicine is used in the management of malaria and inflammatory diseases. Thus its phytoconstituents [flavonoids (FL) and ascorbic acid (AA) isolate] were subjected to *in vitro* immune-stimulatory study at concentration range of 16 – 80 µg/ml. The mean immune-stimulatory index of the FL and AA were compared for statistical significant difference against immunace® (IM) (clinical immune-stimulatory drug – positive control) by one-tailed analysis of variance at P = 0.05. AA [22.98 – 72.07% phagocytosis stimulation, (PS)] and FL [24.95 – 63.64% PS] significantly increased the phagocytosis properties of human neutrophils, with respect to immunace® [34.43 – 92.90% PS], in a dose dependent manner. The observed 50% stimulatory concentrations (SC_{50}) were 35.32, 55.52, and 62.74 µg/ml for IM (positive control), AA and FL, respectively. FL and AA tested positive to 1, 1-diphenylpicryl hydrazyl radical and $KMnO_4$ assays, indicating strong antioxidant properties. Thus ascorbic acid and flavonoids are potent immune-stimulatory principles of *L. owariensis*, acting via antioxidant mechanism. Therefore, the herb is recommended for use as an immune-stimulatory agent, and adjuvant in the management of diseases involving pro-oxidative state and decreased immunity.

PI79 The prospects of fungal endophytes of Nigerian rainforest medicinal plant origin as sources of novel anticancer drug molecules

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The focus of drug discovery is currently shifting more in favour of plant derived microorganisms such as endophytic fungi [1 – 2], which have been found ubiquitous in their host plants. Nigerian medicinal plants, which hitherto have been poorly investigated, could hold enormous potentials as sources of novel fungi endophytes of medicinal importance. Our investigation of 10 medicinal plant species collected from the rainforest zone in Nigeria led to the isolation of 40 pure strains of fungi endophytes. The crude metabolites of these fungi endophytes were screened for cytotoxic activity against mouse lymphoma cell line (L5178Y) and for antimicrobial activity against strains of Gram positive and Gram negative bacteria as well as on some fungal strains. Further chemical investigation of two of the most promising fungi strains *Corynespora cassiicola* isolated from *Gongronema latifolium* leaves and *Xylaria spp* isolated from *Psidium guajava* leaves yielded 19 biomolecules. These included the new corynesidone D, corynether B and corynether lactone A from *Corynespora cassiicola* and the new (*E*) -methyl 3-(4-(3-oxocyclobutyl)phenyl) acrylate, 5,6-dihydro-7-oxo-19, 20- α-epoxycytochalasin C, 18-desoxy-19, 20- α-epoxycytochalasin C and 18-desoxycytochalasin C from *Xylaria spp*. These compounds are currently being investigated for their cytotoxicity against some selected human cancer cell lines and their ability to modulate the chaperoning activity of the Hsp90 chaperoning machine *in vitro*. To the best of our knowledge, this is the first report on the isolation of endophytes from these plant species and the results so far are quite fascinating. **References:** [1] Debbab, A.; Aly, A. H.; Proksch, P. *Fungal Divers.* **2012**; *57*, 45 – 83. [2] Kusari, S and Spiteller M. *Nat. Prod. Rep.* **2011**, *28*, 1203 – 1207.

PI80 New Bisnorsesquiterpenes, sesquiterpene lactone and neolignan glycoside from *Maytenus senegalensis* leaves: structures and cytotoxic activity

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Chemical investigation of the methanol leaf extract of *Maytenus senegalensis* led to the isolation of 16 compounds, including the new maysecyclononeone A, (6S, 9S) 9,10-dihydroxy-4,7Z-megastigmadien-3-one, mayselignoside A and maysefuropyranone A. In addition the following known compounds, viz. (+) lyoniresinol, (-) isolariciresinol, dihydrodehydrodiconiferyl alcohol, (-) epicathechin, (+) gallocatechin, (-) epigallocatechin, procyanidin B-2, 2,3-Dihydrokaempferol 3-O-β-*D*-glucopyranoside, quercetin 3-O-β-*D*-glucopyranoside, kaempferol 3-O-β-*D*- xylopyranoside, 3,5-dimethylgallate and benzoyl maleic acid were also isolated. The structures of the compounds were deduced by a combination of 1 D and 2 D NMR spectroscopy and high resolution mass spectrometry. All compounds were tested for cytotoxicity against mouse lymphoma cell line (L5178Y). Of the 16 compounds only (-) epigallocatechin showed high cytotoxicity and completely inhibited cell growth at the dose of 10 µg/mL.

PI81 Antioxidant phenolics from the leaves of *Alstonia boonei* de Wild

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Alstonia boonei De Wild (Apocynaceae) leaves are used traditionally in West Africa for the treatment of various ailments including rheumatic and muscular pains as well as hypertension and malaria[1]. Chemical investigation of the ethyl acetate fraction of the leaf extract led to the isolation of 10 phenolic compounds. The structures of these compounds were determined using UV, HPLC-MS, 1 D and 2 D NMR spectroscopy and identified as follows: Quercetin-3-O- [α-L-rhamnopyranosyl (1→6)] β-D-glucopyranoside (**1**); quercetin-3-O- [α-L-rhamnopyranosyl (1→6)] β-D-galactopyranoside (**2**); kaempferol 3-O- [α-L-rhamnopyranosyl (1→6)] β-D-glucopyranoside (**3**); kaempferol -3-O- [α-L-rhamnopyranosyl (1→6)] β-D-galactopyranoside (**4**); quercetin-3-O- [α-L-rhamnopyranosyl (1→4)] β-D-glucocpyranoside (**5**); quercetin-3-O- [α-L-rhamno-

pyranosyl (1→4)] β-D-galactopyranoside (**6**); quercetin-3-O- [α-L-rhamnopyranosyl (1→2)] β-D-glucopyranoside (**7**); quercetin-3-O- [α-L-rhamnopyranosyl (1→2)] β-D-galactopyranoside (**8**); chlorogenic acid (**9**) and 3 – 4-dicaffeioylcinnamic acid (**10**). These compounds are being reported for the first time from *Alstonia spp*. Compounds **1**, **2**, **5**, **6** and **9** showed high antioxidant activity on DPPH free radical scavenging model with IC_{50} values of 52, 54, 39, 65 and 49 μg/mL respectively. The two kaempferol derivatives (**3** and **4**) did not show good antioxidant activity (IC_{50} > 100 μg/mL). The antioxidant activity of some of the isolated quercetin derivatives and chlorogenic acid may explain the ethnomedicinal use of the plant extracts in the management of diseases associated with oxidative stress. **References:** [1] Iwu, M.M. 1993. Handbook of African Medicinal Plants. CRC Press, Boca Raton, FL, pp. 116 – 118

PI82 Effect of gamma radiation in fractions end (8*R*,8'*R*,9*S*)-cubebin isolated from the alcoholic extract of the stem bark of *Aristolochia esperanzae* Kuntze

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The species *Aristolochia esperanzae* kuntze is found in Minas Gerais and used in the treatment of Rheumatoid Arthritis by the folk medicine of this region. The herbal drugs have a high level of microbiological contamination, and a alternative to increase the shelf life and reduce microbial contamination of herbal medicines is the gamma irradiation. This paper describes the effect of gamma irradiation in fractions rich in flavonoids and saponins obtained from the alcoholic extract of the stem bark of the species as well as on the fitoconstituinte, (8*R*,8'*R*,9*S*)-cubebin (**Fig. 1**) isolated from the stem bark of *A. esperanzae*.[1]

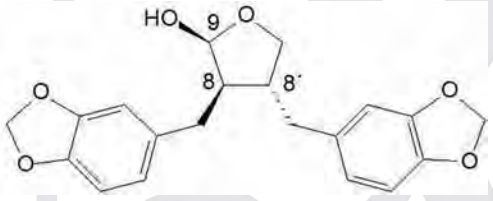

Fig. 1: Structure of (8*R*, 8'*R*, 9*S*)-cubebin.

Sample 0.1 g of (8*R*,8'*R*,9*S*)-cubebin was submitted to gamma irradiation at a dose of 10 kGy. Samples subjected to gamma irradiation and unirradiated were analyzed by NMR. The ^{13}C NMR spectra of (8*R*,8'*R*,9*S*)-cubebin showed different signals, which indicated the formation of radiolytic products after gamma irradiation. The fractions rich in flavonoids and saponins were subjected to gamma radiation at a dose of 0.3 kGy to investigate the integrity front of the gamma irradiation. The irradiated and unirradiated materials was submitted to HPLC analyzes to investigate the effect of gamma irradiation. The chromatograms obtained by HPLC of fractions rich in flavonoids and saponins are shown in **Fig. 2**. Comparison of chromatograms obtained by HPLC analysis of these fractions indicates the integrity of the samples under the conditions considered.

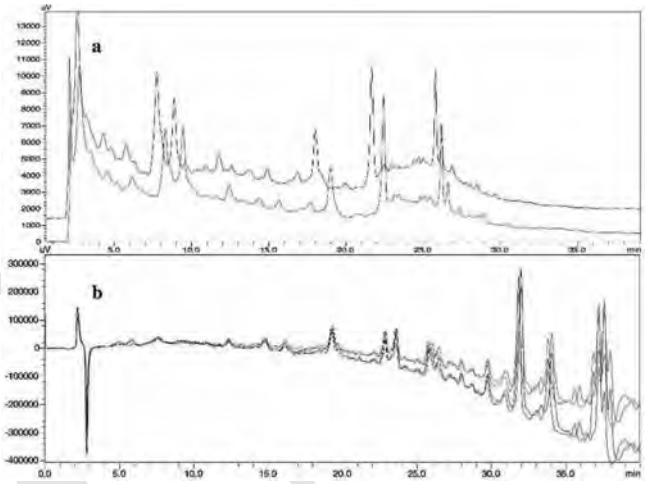

Fig. 2: Comparison of chromatograms obtained by HPLC of fractions rich in flavanoids (a) and saponins (b), (non irradiated –––; subjected to gamma radiation dose of 0.3 kGy –––; the chromatogram: UV detection at 370 nm; chromatogram b: UV detection at 210 nm (–––/–––) and 202 nm (–––/–––).

Acknowledgements: CNPq, UFMG, and IFSULDEMINAS **References:** [1] Pacheco, A. G.; Silva, T. M.; Manfrini, F. M.; Sallum, W. S. T.; Duarte, L. P.; Piló-Veloso, D.; Alcantara, A. F. C. *Quim. Nova*, **2010**, *33*, 91. [2] Silva, T. M. Dissertação de Mestrado, **2010**, Belo Horizonte, UFMG.

PI83 Pure isolation of biologically active compounds and proteins from different plants by adsorptive bubble separation

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Developing biologically active products from medial herbs is of interest to the pharmaceutical industries. For their isolation, methods such as solvent extraction or supercritical fluid extraction are employed. However these methods tend to burden the ecosystem by using organic solvents extensively or demand considerable attention and, hence greater investiment and costs. During the extractions, fat and chlorophyll are co-extracted and, thereafter have to be separated from the active substance by chromatographic methods. Alternative methods of interest are the so- called "Adsorptive Bubble Separation" and "Tweezing Adsorptive Bubble Separation[1)]. Both methods are effective, especially at low concentrations of substances. Furthermore, they use inert gases, which make them mild methods for extracting substances that are sensitive to oxidation. As surfactants, saponine, gelatine and albumine at different pH can be used. These substances can be eliminated after the foam fractionation. During the last ten years, a great number of biologically active compounds such as gingeroles, catechines etc, enzymes such as laccases etc, and hormones for example insulin could be isolated by this way in pure form [2)] By applying tweezing-adsorptive bubble separation, Laccase C and Horseradish Peroxidase could be enriched 10 – 14-fold respectively, without significant loss of enzmatic activities[3)] Also, the enzymes MMP9 and Carboxypeptidase A could be separated with recoveries higher than 85%. The method is based on chelation of enzymes active center with a surface- active chelator such as N-(2-Acetamido)iminodiacetic acid coupled with an octyl- or nonyl- unit. The formed complexes are surface active and can be transferred easly to the foam phase. **References:** [1] M. Backleh-Sohrt, P. Ekici, G. Leupold, and H. Parlar. J. Nat. Prod. 68,1386(2005) [2] A. Nicolai, A. Friess and H. Parlar. J. Sep. Sci.31,2310(2008) [3] BM. Gerken, A. Nicolai, D. Zorn, RG. Berger and H. Parlar. Anal. Chem77,6113(2005)

PI84 Fungal transformation of diterpenes by *Aspergillus phoenix*

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Microbial transformation is an interesting tool used to increase the variety of chemical structures to be applied in the search for novel bioactive compounds [1]. In present work, the biotransformation of kaurenoic acid (**1**) and copalic acid (**4**), two diterpenes isolated in high amounts from Brazilian plants (*Mikania glomerata* and *Copaifera langsdorffii*), were performed using submerged liquid culture of *Aspergillus phoenix* (3,76 X 10^8 esporos/mL). The microorganism was grown by a two-stage fermentation procedure. Substrate were added as a dimethylsulfoxide solution (0.1 g/L) and incubated for 7 days. The cultures were filtered and the aqueous layers were extracted with ethyl acetate to furnish the extracts codified as AC (obtained from **1**) and Aco (obtained from **4**). Chemical and NMR studies of AC and Aco allowed us to isolate and to identify four hydroxylated derivatives. Compounds **2** and **3** were isolated from AC, while **5** and **6** from Aco. Both antimicrobial and antitumor activities of such compounds will be investigated by our research group. **Acknowledgements:** FAPESP (Proc. 2011/21638 – 8 and 2011/13630 – 7), CNPq and CAPES. **Reference:** [1] Marquina S, et al. (2009) Phytochemistry 70: 2017.

PI85 Neuritogenic activity of coumarins from *Clausena harmandiana*

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Coumarins have been identified from natural sources, especially green plants such as *Clausena harmandiana*. Antioxidant activity of the plant was assayed using 1,1-diphenyl-2-pricrylhydrazyl (DPPH) and Thiobarbituric Acid Reactive Substances (TBARS) methods while its neuritogenic effects was assessed on cultured P19 neurons. Nordentatin, isolated from the root bark of *C. harmandiana*, showed more potent antioxidant and inhibition of lipid peroxidation than its derivatives, dentatin and xanthoxyletin. Nordentatin with IC_{50} > 10 mM and viability of 82.74 ± 24.54% showed no neurotoxicity while dentatin and xanthoxyletin expressed neurotoxicity at 1 nM (viability 62.28 ± 12.11% and 43.02 ± 10.31%, respectively). Phase-contrast micrographs showed that nordentatin promoted the outgrowth of the neurites of the neurons. The neurons treated with the extracts displayed significantly more branching numbers of the neurites than the control ($p < 0.05$), indicating that nordentatin was a remarkable natural neuritogen.

PI86 Isolation and quantification of alkaloids from *Tetrapterys mucronata* – a plant used in the ayahuasca preparation

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Tetrapterys mucronata Cav. (Malpighiaceae) is a plant used in some regions of Brazil in ayahuasca preparation a psychotropic plant decoction [1]. To assess the phytochemical composition of *T. mucronata* and understand its traditional use, aqueous and methanolic extracts were investigated. Their HPLC-PDA-ESI-MS profiles have been determined. The isolation of the main compounds of the methanolic extract was performed by a direct transfer of analytical HPLC conditions to medium pressure liquid chromatography (MPLC). This resulted in the isolation of six alkaloids and one new phenanthrene derivative. Since tryptamine and β-carboline alcaloids are known to have powerful hallucinogenic activities, an HPLC-ESI-MS/MS method has been developed for their quantification in *T. mucronata* water and methanolic extracts. These results provide a rational support for the traditional use of *T. mucronata* stem bark in ayahuasca preparations, since β-carboline alkaloids are present in a relevant amount. **Reference:** [1] Ott, J., *Ayahuasca analogues: Pangæan entheogens*. 1st ed.; Natural Products Co.: Kennewick, WA, **1994**; p 127.

PI87 Innovative strategies for the efficient isolation of natural at the preparative scale

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In natural product research the isolation of compounds at the milligram scale is key to assess their bioactivity *in vivo*. Conventional isolation approaches are often time-consuming and employ relatively complex schemes that involve different preparative chromatographic methods. One efficient strategy consists in the transfer of extract profiling gradient conditions from HPLC to semi-prep HPLC [1]. In order to further increase the sample loading, Medium Pressure Liquid Chromatography (MPLC) represents an interesting alternative since grams of crude extract can be separated in one step and we have developed models for accurate gradient HPLC-MPLC transfer. MPLC separations are however limited by the maximal flow rate and the pressure allowed by the glass columns used and implies that the separations are carried out over several days. To overcome this problem the pressure drop was minimised by designing a dedicated column oven for preparative separation and perform separations under relatively high temperatures. The speed of separation was enhanced by maximising the flow and using a new way of controlling pressure at the column inlet. For a comprehensive survey of the isolation, MPLC fractions were also monitored post-chromatographically by ultra-fast UHPLC/TOF/MS and provided 2D LC x LC matrices that contain all information enabling an optimal fraction combination. The improvements of separation obtained are illustrated with the separation of different crude plant extracts each containing different classes of molecules. In these different cases a majority of pure NPs could be obtained in mg amounts in about one to two days. **Reference:** [1] Glauser G, Guillarme D, Grata E, Boccard J, Thiocone A, Carrupt P-A, Veuthey J-L, Rudaz S, Wolfender J-L, *J Chrom A* **2008**, 1180, 90.

PI88 Extract of maydis stigma (*Zea mays* L.) inhibits the adhesion of uropathogenic *Escherichia coli* (UPEC) to human bladder cells

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The stigma/style of the female flower of *Zea mays* L. (Poaceae) has been used traditionally as a therapeutic remedy for various ailments such as cystitis, urethritis, nephritis, nocturnal enuresis, prostatitis, gout, and kidney stones in form of decoctions or aqueous-alcoholic extracts for per oral use. Due to literature the stigmata contain proteins, vitamins, carbohydrates, fixed and volatile oils, phytosterols, alkaloids, saponins, tannins, and flavonoids. Therefore maydis stigma was screened on potential antiadhesive activity against UPEC by an *in vitro* assay with a positive outcome (IC_{50} 1041 µg/mL) [1]. The present study was carried out to determine responsible compounds from maydis stigma against UPEC. 1 kg of dried plant material (Hungarian origin) was extracted by Soxhlet with petroleum benzine for 6 h. The residue was air dried at room temperature for 12 h and then reextracted with 10 L EtOH/water (1:1) by ultraturrax. The extract was fractionated by gel permeation chromatography on Sephadex LH-20. The received 22 fractions were investigated on potential antiadhesive activity with FITC-labeled UPEC on T24 bladder cells by flow cytometric evaluation. Fraction I (approx. IC_{50} 1218 µg/mL) and XI (approx. IC_{50} 1120 µg/mL) showed strong antiadhesive activity. Due to agar diffusion assay and MTT assays direct cytotoxicity against UPEC and T24 cells can be excluded. From fraction I a Dragendorff-positive compound was isolated by extraction with CH_2Cl_2 from the alkaline solution. The respective steroidal alkaloid ($C_{27}H_{41}NO_2$ after ESI MS, $\lambda_{max.}$ 210 nm) showed strong antiadhesive activity and has to be regarded as the main active principle of the herbal material. **Reference:** [1] Rafsanjany N, Lechtenberg M, Petereit F, Hensel A (2013) Antiadhesion as a functional concept for protection against uropathogenic *E. coli*: *in vitro* studies with traditionally used herbal extracts as antiadhesive entities against uncomplicated urinary tract infections. J. Ethnopharm. 145, 591 – 597

PI89 Identification of dihydrostilbenes as a new scaffold for $GABA_A$ receptor modulators in *Pholidota chinensis* stems and roots

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In a search for new natural product-derived $GABA_A$ receptor modulators, we screened a plant extract library on *Xenopus laevis* oocytes expressing recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors, by means of a two-microelectrode voltage clamp assay. A dichloromethane extract of stems and roots of *Pholidota chinensis* (Orchidaceae) enhanced the GABA-induced chloride current (I_{GABA}) by 132.75%± 36.69% at 100 µg/mL. By means of an HPLC-based activity profiling approach, the three structurally related stilbenoids coelonin (**1**), batatasin III (**2**), and pholidotol D (**3**) were identified. Dihydrostilbene **2** enhanced I_{GABA} by 1512.19%± 176.47% at 300 µM, with an EC_{50} of 52.51 ± 16.96 µM, while compounds **1** and **3** showed much lower activity, suggesting conformational flexibility as in **2** to be crucial for receptor modulation. This was confirmed by a study on a series of 11 commercially available stilbenoids and their corresponding semisynthetic dihydro derivatives. When tested at a concentration of 100 µM, dihydrostilbenes showed higher activity in the oocyte assay than their corresponding stilbenes. The dihydro derivatives of tetramethoxy piceatannol (**6**) and pterostilbene (**11**) were the most active, with modulations comparable to that of compound **2** (544.5%± 104.4% and 660.6%± 100.2%, respectively), when tested at the same concentration. Dihydrostilbenes represent a new scaffold for $GABA_A$ receptor modulators.

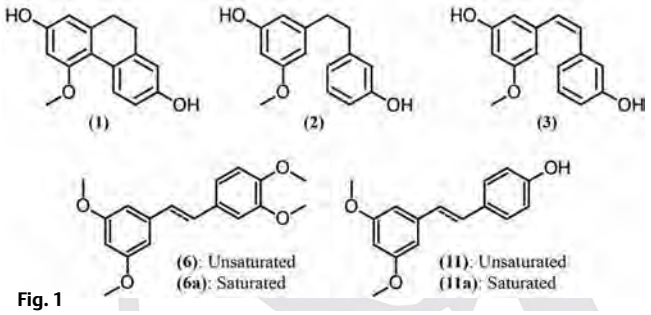

Fig. 1

PI90 Isoflavonoid derivatives from *Adenocarpus cincinnatus* as positive $GABA_A$ receptor modulators

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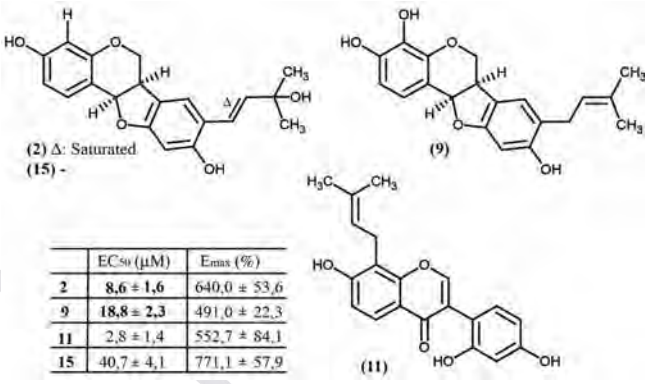
$GABA_A$ receptors are the major inhibitory receptors in the CNS, and the target for benzodiazepines, barbiturates, and various other CNS depressants. Such drugs, however, lack selectivity for different $GABA_A$ receptor subtypes and thus, the pharmacological treatments of anxiety, epilepsy, insomnia, and mood disorders are accompanied by serious side effects. Consequently, the search for selective modulators with new scaffolds is still an urgent task. In a search for new natural product-derived $GABA_A$ receptor modulators, we screened a library of 880 plant and fungal extracts by means of a two-microelectrode voltage clamp assay, with *Xenopus laevis* oocytes expressing recombinant $GABA_A$ receptors of the subtype $\alpha_1\beta_2\gamma_{2S}$. A dichloromethane extract of *Adenocarpus cincinnatus* roots (Fabaceae) enhanced the GABA-induced chloride current (I_{GABA}) by 126.5%± 25.1% at 100 µg/mL. By means of HPLC-based activity profiling, a known 8-prenylisoflavone (**10**) and three new structurally related *cis*-pterocarpans (**2**, **8**, **14**) were identified as responsible for the activity. They showed promising activity in the oocyte assay, potentiating I_{GABA} by 491.0% to 771.1%, and exhibiting EC_{50} values ranging from 2.8 µM to 40.7 µM. Further isoflavonoid derivatives, including the known *cis*-pterocarpan maackiain, were isolated from the extract and tested in the oocyte assay. These compounds show lower but still interesting activities, with I_{GABA} enhancements ranging from 100% to 300%. This work reveals pterocarpans as a new scaffold for $GABA_A$ receptor modulators.



	EC_{50} (µM)	E_{max} (%)
2	8,6 ± 1,6	640,0 ± 53,6
9	18,8 ± 2,3	491,0 ± 22,3
11	2,8 ± 1,4	552,7 ± 84,1
15	40,7 ± 4,1	771,1 ± 57,9

Fig. 1

PI91 Jatrophane diterpenoids with multidrug resistance-modulating activity from *Euphorbia exigua*

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A primary mechanism of resistance to multiple anticancer drugs is the overproduction of permeability glycoprotein (P gp) in plasma membranes of resistant cells. The P gp acts as an energy-dependent efflux pump, reducing the intracellular accumulation of drug molecules. In recent years, considerable attention has been devoted to the development of new effective multidrug resistance (MDR) modulating agents from natural sources. Promising group of anti-MDR molecules are the macrocyclic diterpenes from Euphorbiaceae species. We report herein the isolation and structure determination of diterpenes from *Euphorbia exigua* L., an annual weed with Southern-temperate distribution, whose diterpene constituents has not been reported previously. The methanol extract of the fresh aerial parts of *E. exigua* was subjected to solvent partitioning to furnish chloroform- and water-soluble fractions. The organic phase was fractionated by column chromatography on polyamide, then by vacuum liquid chromatography on silica gel. Selected fractions were further purified by CPC, preparative TLC and HPLC to yield three pure compounds. The structure elucidation was carried out by HRESIMS and extensive NMR studies using advanced experiments (^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, HSQC and HMBC). The isolated compounds were identified as jatrophane polyesters. Two of them are new natural compounds acylated with acetic, benzoic, propanoic, angelic and cinnamic acids. One compound proved to be identical with isoterracinolide B isolated earlier only from *Euphorbia terracina*. The investigation of the compounds for modulating intracellular drug accumulation of MDR mouse lymphoma cells resulted that all three compounds promoted the accumulation of rhodamine 123 at a level similar to the parental cell line which do not over-express the MDR1 pump. This work was supported by European Union and co-funded by the European Social Fund (TÁMOP-4.2.2.A-11/1/KONV-2012 – 0035) and Hungarian Scientific Research Fund (OTKA) (PD 78145).

PI92 Polar secondary metabolites from *Ocimum sanctum* L.

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The aim of this study was to investigate the polar constituents of *Ocimum sanctum* L. (Lamiaceae), which is used in *ayurvedic* medicine under the common name *tulsi*. In continuation to our previous studies [1 – 3], we now investigated the methanol: water (7:3) extract of its aerial parts. The extract was fractionated using RP_{18}-MPLC, CC on Sephadex LH 20 (MeOH) and semi-prep RP_{18}-HPLC and afforded *trans*-p-coumaric acid 4-O-β-D-glucopyranoside; 3-(3,4-dihydroxyphenyl)lactic acid; protoca-

techuic acid; (-)-rhabdosin, a caffeic acid tetramer with a lignan skeleton; the neolignan shimobashiric acid C, recently isolated from *Keiskea japonica*-Lamiaceae [4]; apigenin 7-O-β-D-glucuronide and luteolin 7-O-β-D-glucuronide. The structures of the isolated compounds were confirmed by spectroscopic methods, including 1D- & 2D-NMR, UV-Vis. Our results corroborate with literature concerning the flavonoid pattern. The genus *Ocimum* L. is divided in three subgenera: subgenus *Ocimum* (sections Ocimum, Gratissima and Hiantia), subgenus *Nautochilus* and subgenus *Gymnocimum*. *O. sanctum* L. belongs to the subgenus *Gymnocimum*, which is characterized by the presence of flavonoid glucuronides, totally absent in the taxa of the other two subgenera [5]. **References:** [1] Skaltsa H., Couladi M., Philianos S., Singh M., 1987. Fitoterapia, LVIII, 4, 286; [2] Skaltsa H., Tzakou O., Singh M.., 1999. Pharmaceutical Biol., 37, 92 – 94; [3] Skaltsa H., Tzakou O., Loukis A., Argyriadou N., 1990. Plant. Méd. Phytoth., XXIV, 2, 79 – 81.; [4] Murata T, Miyase T, Yoshizaki F., 2012. Chem. Pharm. Bull. 60, 121 – 128; [5] Grayer RJ, Kite GC, Veitch NC, Eckert MR, Marin PD, Senanayake P, Paton AJ., 2002. Biochem. Syst. Ecol., 30, 327 – 342.

PI93 New neolignans from leaves of *Miliusa mollis*

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Miliusa mollis Pierre (Annonaceae) is a shrub found in the northern and central regions of Thailand where it is locally known as Ching-chap [1]. Recently, the presence of neolignans, aporphine alkaloids and phenolic glycoside in the twigs of *M. mollis* has been reported [2]. In this study, we report the isolation of six new neolignans from the leaves of this plant including miliumollin (**1**), 4'-*O*-methylmiliumollin (**2**), 3'-methoxymiliumollin (**3**), 7-methoxymiliumollin (**4**), miliumollinone (**5**) and miliusamollin (**6**), and a known compound named decurrenal (**7**). Their structures were elucidated through analysis of their spectroscopic data. Four neolignans (**1**, **3**, **5** and **7**) exhibited weak cytotoxic against KB, MCF7 and NCI-H187 cells, but only **5** showed weak inhibitory effects against herpes simplex virus types 1 and 2 (HSV1 and HSV-2).

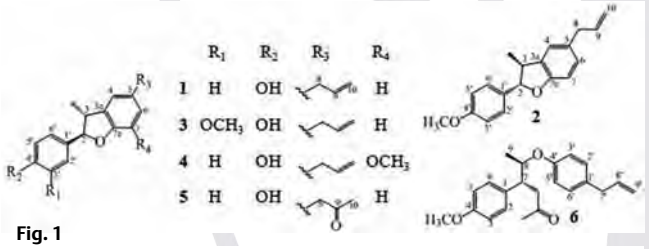

Fig. 1

Acknowledgements: The Thailand Research Found in the form of a Royal Golden Jubilee Ph.D. scholarship (PHD/0261/2549), The French Embassy in Thailand for an RGJ-matching grant, Chulalongkorn University Graduate Scholaship in Commemoration of HM King Bhumibol Adulyadej's 72nd Anniversary **References:** [1] Smitinand, T. (2001) Thai Plant Names, revised Ed. Prachachon Co. Ltd. Bangkok, Thailand. [2] Sawasdee, K. et al. (2010) Molecules 15: 639 – 648.

PI94 Thiolysis-HPLC/MS characterization of oligomeric and polymeric proanthocyanidins in *Ephedra sinica*

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Proanthocyanidins (PAs) can be found in a wide variety of edible plants such as green tea, cranberry and peanuts. They have been reported to have anticancer, antiallergical and antimicrobial activity and are effective antioxidants. *Ephedra sinica* Stapf (Ephedraceae), a plant widely used in TCM, is known to contain PAs, but so far only unusual dimeric PAs, e.g. ephedrannin A or B, have been identified as constituents of this plant [1]. The aim of this work was the characterization of oligomeric and polymeric PAs from aerial parts of *E. sinica*. By means of silica gel column chromatography and precipitation from methanol and chloroform mixtures, PA enriched fractions varying in their degree of polymerization were obtained. Samples were analyzed by a thiolytic degradation/HPLC-MS assay. Reference compounds were prepared by thiolytic degradation of a larger amount of *Ephedra*-PAs followed by isolation and structure elucidation via LC-MS and NMR. Analysis of the precipitated fractions revealed a mean degree of polymerization between 15 (precipitate 50% methanol) and 6 (precipitate 25% methanol). Monomeric units were identified as gallocatechin and epigallocatechin (81 – 83% of total units) as well as catechin and epicatechin. Interestingly, the investigated PAs turned out to be a complex mixture of double linked A-type and single linked B-type units. Results of this study suggest that aerial parts of *E. sinica* are a useful source of oligomeric and polymeric PAs mainly consisting of epigallocatechin and gallocatechin monomers. Studies to investigate the contribution of these compounds to the pharmacological activity of *E. sinica* are ongoing. **References:** [1] Tao et al.(2008) Planta Medica 74: 1823 – 5

PI95 Unsaturated fatty acids are potent MHC-II loading enhancers (MLE)

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Small molecules that enhance the binding of antigens to MHC-II molecules are (MHC-loading enhancers, MLE) may be involved in the pathophysiology of autoimmune diseases. They may also be relevant to potentiate the efficacy of vaccines and immunotherapeuticals [1]. We recently reported on the MLE activity of terpenoids as well as acetylenic spiroethers from essential oils [2]. In search for further polyynes with MLE activity we have now investigated the CH_2Cl_2 extracts of underground parts of the Apiaceae *Foeniculum vulgare* var. *azoricum* (Fennel), *Pastinaca sativa* ssp. *sativa* (Parsnip) and *Apium graveolens* var. *rapaceum.* (Celery), which displayed interesting results in our test system (Dissociation-enhanced fluorescent immunoassay measuring loading of soluble MHC-II (HLA-DR1) with an influenza HA peptide in comparison with the positive control Adamantylethanol [1, 2]). Bioassay-directed fractionation of the root extracts showed the highest activity in fractions containing unsaturated fatty acids (FA). LC/MS and NMR analyses revealed linoleic acid (LA) as constituent in the most active fractions. Consequently, a variety of natural FA were tested for MLE activity (Table 1). Overall, ALA was the most active FA (Fig. 1). Other unsaturated FA were markedly less active. Saturated FA did not show any MLE activity. Moreover, Linseed oil, rich in ALA, was inactive. The free acid hence must be the active principle directly responsible for MLE activity. The finding that particular unsaturated FA, important constituents of daily nutrition possess a specific MLE effect represents a novel aspect regarding their physiological importance. These FA – besides nutritional value and involvement in homeostasis, membrane integrity and mediator synthesis – may thus also be involved in processes directly related to antigen presentation and immunity.

Tab. 1: n-fold increases of the absolute MLE-activity of fatty acids in comparison to 1-Adamantylethanol

Unsaturated fatty acid	n at c = 0.01%	n at c = 0.002%
Oleic acid	26.7	18.2
α-Linolenic acid	56.1	22.3
γ-Linolenic acid	37.9	18.6
Arachidonic acid	12.7	8.0
Ricinoleic acid	21.9	9.7
1-Adamantylethanol*	71	6.5
Linoleic acid	18.3	11.4
1-Adamantylethanol*	37.9	4.1
*Positive control measurements of two independent experimental series		

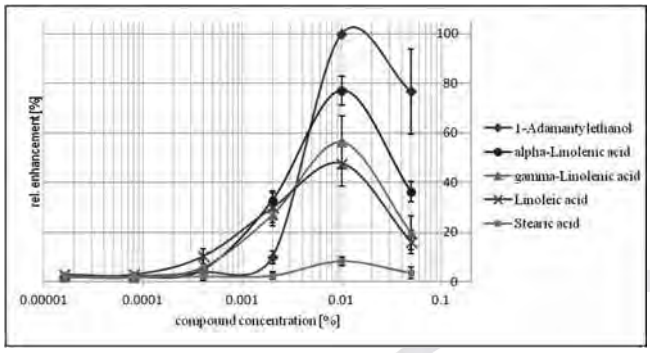

Fig. 1: MLE-activity of fatty acids in relation to the control 1-Adamantylethanol

References: [1] Höppner S et al., 2006. J. Biol. Chem. 281: 38535 – 42. [2] Schnieders A et al., 2011, Planta Med. 77, PM174

PI96 New constituents of the seeds of *Sesamum indicum* L.

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The seeds of *Sesamum indicum* L. are a well-known source of lignan-derivatives. The aim of the performed study was to isolated reference compounds for the development of an analytical method. The isolation of polar constituents (*n*-butanol soluble) of an extract of unpeeled sesame seeds (defatted with *n*-hexane, extracted with methanol) resulted in the isolation of five known lignan-derivatives: (+)-pinoresinol-4,4'-*O*-β-D-diglucopyranoside; (+)-pinoresinol-4-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside; sesaminol-2-*O*-β-D-glucopyranosyl-(1→2)-*O*-[-β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside; sesamolinol-4-(*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside; sesaminol-2-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside. Beside these known compounds, the performed isolation reveled two new lignan-derivatives: (+)-episesaminon-9'-*O*-β-D-glucopyranosyl-(1→2)-*O*-[-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosid and (-)-sesaminon-9'-*O*-β-D-glucopyranosyl-(1→2)-*O*-[-β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside and a novel alkaloid with an unusual tricyclic skeleton which was named (-)-sesam-indol-N-oxide. Structures were elucidated by 1- and 2-D-NMR spectroscopy and LC-MS^n experiments.

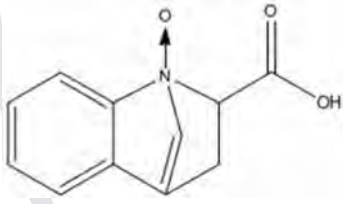

Fig. 1

PI97 Novel anti-inflammatory neolignans and dineolignans from the fruit of *Magnolia obovata*

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Introduction and Objective: *Magnolia obovata* (Magnoliaceae), a deciduous tree, growing up to 20 m high and is widely distributed in Korea, China, and Japan. It contains many kinds of secondary metabolites such as neolignans, sesquiterpene-neolignans and flavonoids. These compounds were reported to show anti-gastric, anti-platelet, and anti-complement activities However, studies on the fruits of *M. obovata* barely investigated for its chemical constituents and biological activities. Therefore, the present study focused on isolation and identification of active constituents in the fruit of this plant, as well as examination of the pharmacological activities of the isolated compounds. **Methods:** The fruit of *M. obovata* were extracted with 80% aqueous MeOH, and the concentrated extract was partitioned with EtoAc, *n*-BuOH, and H_2O, successively. From the EtOAc fraction, four new neolignans and 6 new dineolignans along with six known ones were isolated through the repeated SiO_2, ODS column chromatografies. **Results:** Our phytochemical study on the fruit of *M. obovata* led to isolation and identification of four new neolignans **3**, **7**, **8**, **10** as well six known ones **1**, **2**, **4**-**7**, **9** and six new dineolignans **11**-**16** by spectroscopic analysis including ^{1}H and ^{13}C-NMR, DEPT, and 2D-NMR. These ten neolignans and six dineolignans were identified as magnolol (**1**), honokiol (**2**), isoobovatol (**3**), isomagnolol (**4**), obovatol (**5**), obovatal (**6**), 9-methoxyobovatol (**7**), magnobovatol (**8**), obovaaldehyde (**9**), 2-hydroxyobovaaldehyde (**10**), and new dineolignan (**11**-**16**). **Conclusion:** All isolated compounds **1**, **2**, **4**, **5**, **7**, **8**, and **10** inhibited nitric oxide production in RAW 264.7 cells with IC_{50} values of 15.8 0.3, 3.3 1.2, 12.9 2.0, 6.2 1.2, 14.8 2.3, 14.2 1.2, and 14.8 3.2 μM, respectively, without obvious toxic effect.

PI98 Characteristics of low molecular weight extractives from *Prunus avium* seeds

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Cherry of *Prunus avium* (Rosaceae) is one of the most popular temperate fruits. It is very attractive to consumers due to its taste, sweetness and wealth of nutrients. Moreover, *P. avium* cherry is considered to be a rich source of bioactive natural compounds, which can act as powerful antioxidative agents and reported to have many health promoting effects, including antitumor, anti-inflammatory and antibacterial activities [1]. *P. avium* fruits can also used as an ingredient in fruit cocktails and in the manufacture of confectionery fillings, as well as for so-called maraschino cherries [2]. Previous phytochemical investigation on *P. avium* were mainly concentrated on its cherries. However, to date, chemical composition of seeds of *P. avium* has never been carried out yet. In the current work, six low molecular weight extractives were isolated and purified from *n*-BuOH fraction of 95% aqueous methanol extracts of *P. avium* seeds by Sephadex LH-20 and silica gel open column chromatography coupling with TLC systematically. Sturctures of the six isolated compounds were elucidated through extensive spectroscopic techniques and sorted as two flavan-3-ols [(+)-gallocatechin (**1**) and (-)-epicatechin (**2**)], one flavonol [kaempferol (**3**)], one flavonoid glycoside [apigenin-7-*O*-β-D-glucopyranoside (**4**)], and two phenolic acids [gallic acid (**5**) and caffeic acid (**6**)]. Among these low molecular extractives, compounds **1**, **4**, **5** and **6** were isolated for the first time in genus of *Prunus*. **References:** [1] Serra AT. et al. (2011) Food Chem 125: 318 – 325. [2] Gao L, et al (1995) J Agric Food Chem 43: 343 – 346. **Acknowledgements:** This work was financed by National Natural Science Foundation of China (31170541, 31000279), Program for New Century Excellent Talents in University (NCET-10 – 0951), Natural Science Foundation of Tianjin City (13JCZDJC), Foundation (2012IM002) of Key Lab of Industrial Fermentation Microbiology of Ministry of Education & Tianjin Key Lab of Industrial Microbiology, China.

PI99 Isolation and structural elucidation of novel natural compounds from twigs of *Pinus banksiana*

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Pinus banksiana (Pinaceae), also called Jack pine, is an evergreen and fast-growing conifer, with a sparse, variable crown and spreading

branches at maturity. The species is widely distributed in subpolar and polar areas of northern hemisphere, including Taibai, Daxing'an and Xiaoxing'an Mountains of China, northwest territories of America and many forestry regions of Canada [1]. The conifer has long been used in traditional medicines to enhance cardiovascular functions, improve immunity, cure and prevent cancer and aging diseases [2]. Literatures reported that extracts of wood knot of *P. banksiana* exhibited significant anti-tumor and antioxidative activities to human cells [3]. In the current work, phytochemical investigation of *P. banksiana* twigs led to the isolation and purification of five known phenolics and a new lignan derivatives named phillygenin-4-rutinoside (Fig.1). The structures of the new and known natural compounds were extensively elucidated and determined by their NMR and mass spectral evidence and a careful comparasion with authentic compounds. To the best of our knowledge, the six secondary metabolites were found in *P. banksiana* for the first time.

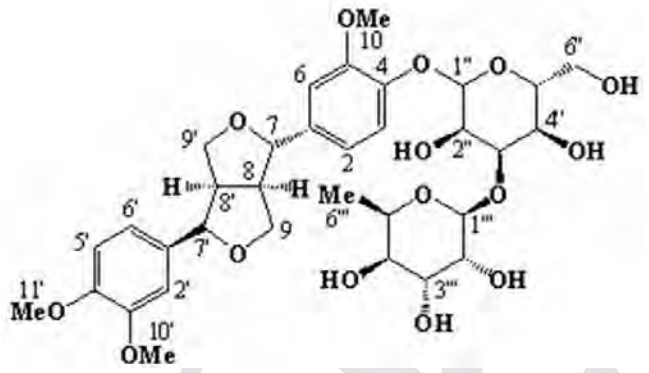

Fig. 1: Phillygenin-4-rutinoside

References: [1] Fu LK, et al. (1999) Flora of China 4: 11 – 25. [2] Liu ML, et al. (2011) J Anhui Agri Sci 39: 15420 – 15421. [3] Phelan M, et al (2009) J Med Food 12: 1245 – 1251. **Acknowledgements:** This work was funded by National Natural Science Foundation of China (31170541, 31000279), Program for New Century Excellent Talents in University (NCET-10 – 0951), Natural Science Foundation of Tianjin City (13JCZDJC), and Foundation (200301) of Jiangsu Provincial Key Laboratory of Pulp and Paper Science and Technology, Nanjing Forestry University, China.

PI100 Natural compounds from *Paulownia tomentosa* var. *tomentosa* root and their antioxidant effects

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Oxidation is critical for the survival of most living organisms in order to obtain the energy needed for biological processes during aerobic respiration. However, excessively high level of oxidation can attack biological macromolecules, and trigger chemical chain reactions such as lipid peroxidation or oxidize DNA or proteins, thereby altering several signaling pathways that ultimately promote cellular damage and death [1]. Due to the safety and the limitation of synthetic antioxidants, the isolation and identification of new antioxidants from natural sources have attained a special interest. *Paulownia tomentosa* var. *tomentosa (PTT)* (Scrophulariaceae) is a tree native to China and distributed throughout eastern Asia [2]. In folk medicines, the root, fruit, leaf, and bark of *PTT* are used to prevent or treat various disorders including asthma, bronchitis and tonsillitis [2]. In the current work, phytochemical investigation of 95% EtOH extractives of *PTT* root, using repeated silica gel and Sephadex LH-20 column chromatography, resulted in the isolation of 6 phenylpropanoid glycosides, including cistanoside F (**I**), campneoside I (**II**), campneoside II (**III**), isocampneoside II (**IV**), verbascodide (**V**) and isoverbascoside (**VI**). The structure elucidation of the isolates was based on their spectroscopical and physiochemical data. This was the first time to report natural compounds of *PTT* root. By ABTS and DPPH radical scavenging assays, **I** and **III**~**VI** (IC_{50} ranged 5.9~6.7 μM on DPPH and 1.0~2.1 mM on ABTS) of *PTT* exhibited significant antioxidant effects, comparing with vitamin E (IC_{50} 6.8 μM and 0.8 mM on DPPH and ABTS, respectively). **References:** [1] Valko M. et al (2005) Curr Med Chem 12: 1161 – 208. [2] Si CL. et al (2013) Bioresources 8: 628 – 637. **Acknowledgements:** This work was supported by National Natural Science Foundation of China (31000279, 31170541), and Foundation (200301) of Jiangsu Provincial Key Laboratory of Pulp and Paper Science and Technology, Nanjing Forestry University, China.

PI101 Secondary metabolites of marine algae *Entermorpha prolifera*

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Marine plants have attracted increasing attention in the search for natural compounds to develop new medicinal and functional food ingredients. *Enteromorpha prolifera* (Ulvaleae), a large green algae, has been reported to exhibit significant antioxidant, antiinflammatory and antitumor activity. In addition *Enteromorpha* species have been used as pharmaceutical product and healthcare food for millennia in Asian countries [1]. In recent years *E. prolifera* has been frequently involved in terrible algal proliferation in China's Qingdao coastal areas. High quantity of *E. prolifera* wrack accumulated along shorelines and on the beaches produced smelly odors and arose environmental issues concerning [2]. Therefore, our current work was carried out to investigate the chemical constituents of this environmental pollutant green seaweed for the achievement of potential high-value-added products. *E. prolifera* was harvested, air-dried and extracted with 95% EtOH. Then the extracts were successively partitioned with polar solvents and freeze dried. Repeated column chromatography on a portion of ethylacetate soluble powders guided by TLC resulted in the purification of four secondary metabolites from *E. prolifera*, and their structures were elucidated as pheophorbide A (**I**), cholesterol (**II**), carotenoid (**III**) and pheophytin A (**IV**), based on extensive spectroscopic techniques such as ^1H NMR, ^{13}C NMR and mass spectrometry. This was the first time to isolate **III** and **IV** from *E. prolifera*. **References:** [1]. Yasuji O. et al. (1997) Int J Immunopharmac 19: 355 – 388. [2]. Liu F. et al. (2013) Mar Environ Res 83: 38 – 47. **Acknowledgements:** This work was financially supported by National Natural Science Foundation of China (31170541, 31000279), Program for New Century Excellent Talents in University (NCET-10 – 0951), Natural Science Foundation of Tianjin City (No. 13JCZDJC), and Foundation (201204) of Tianjin Key Lab of Marine Resources & Chemistry, Tianjin University of Science and Technology, China.

PI102 High-performance counter-current chromatography separation of extract from *Peucedanum cervaria* towards the isolation of coumarin derivatives

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The genus *Peucedanum*, belonging to the Apiaceae family comprises numerous species widely distributed in Europe. Some of these plants are used in traditional medicine for a long time, for the treatment of different serious diseases such as asthma, angina, epilepsy or gastrointestinal disorders. *P. praeruptorum* is officially listed in Chinese Pharmacopoeia. Major bioactive components were identified as angular dihydropyranocoumarins, thus their separation and purification is a very important task. In present research a high-performance counter-current chromatography (HPCCC) method was applied. Method, as an efficient preparative technique, offers a series of advantages over conventional chromatography like elimination of irreversible adsorption of sample or easy scaling-up. HPCCC method was applied for the preparative separation and purification of major coumarins from the fruits of *Peucedanum cervaria*. The Spectrum HPCCC (Dynamic Extractions, UK) equipped with both analytical and semi-preparative columns (22 ml and 137 ml volume, respectively) was used in this study. A scale-up process from analytical to preparative in a very short time was developed. Several

solvent systems: mixtures of *n*-hexane, ethyl acetate, methanol and water in reverse phase system were elaborated. Both rotation speed and flow rate were optimized. After injection of 500 mg of extract four pirano-coumarins were isolated in pure form (4.58 mg; 3.7 mg; 3.14 mg and 1.5 mg) and then analyzed by HPLC-DAD and HPLC/DAD/ESI-TOFMS with accurate mass measurements. Further complete structure elucidation with NMR is in progress. *Acknowledgments:* This work was financially supported by Grant no N N405 617538.

PI103 Antibacterial activity of different extracts of *Daucus carota* canopy

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Genus *Daucus* is a member of Apiaceae family which is one of the most important families of angiosperms. The usage of these plants, particularly as flavourings, spices, and in traditional medicine, is connected with aromatic compounds that may occur in all parts of the plant. Carrots (*Daucus Carota*) are nutritional heroes; they store a goldmine of nutrients; e. g. carotene as carrots, which the body converts it to vitamin A, an excellent source of vitamins B, C, D and E, thiamine, folic acid and magnesium, as well as calcium pectate (pectin fibre) which have cholesterol-lowering properties. They are used in the diet of cancer patients, the dried flowers are used as a remedy for dropsy, besides, they are antianaemic, healing, diuretic, anthelmintic, carminative, emmenagogue, ophthalmic and sedative properties. Its phytochemical screening showed the presence of coumarin, anthocyanins, phenolic acids, flavones, flavanols and their glycosides and terpenes In the present study, dried collected *Daucus Carota* canopy was powdered then extracted by successive solvent extraction in Soxhlet extractor with chloroform followed by ethyl acetateand finally ethanol. The three extracts were vacuum dried and checked for their antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans and Escherichia coli*. The ethanolic extract gave the largest zone of inhibition i. e. showed to be the most active one against the tested bacteria. Thus, phytochemical screening of this extract was carried out and indicated the presence of apigenin 7-O-β-D-arabinoside-4'-O-β-D-glucoside, 7-O-β-D-glucoside, 4'-O-β-D-glucoside, 7-O-β-D-arabinoside, luteolin 7-O-β-D-mannoside-4'-O-β-D-glucoside, 7-O-β-D-galactoside, 7-O-β-D-mannoside, chrysoeriol 7-O-β-D-arabinoside, 7-O-β-D-glucoside besides the three aglycones: luteolin, apigenin, chrysoeriol and the following phenolic acids: caffeic, chlorogenic, and ferulic acids.

PI104 Mycosporine-like amino acids (MAAs): qualitative analysis of photoprotective compounds in *Laurencia* complex seaweed species (Ceramiales, Rhodophyta) from Espírito Santo State, Brazil

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Sessile and sedentary marine organisms that occupy the light-intense environment of coral reefs and rocky shores, such as benthic algae, have evolved physicochemical strategies to reduce ultraviolet (UV) damage. One of these strategies is the synthesis of mycosporine-like amino acids (MAAs), molecules characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid with UV-absorption maxima in the range 309 – 362 nm. MAAs are widely believed to act as sunscreens against biologically damaging UVB (280 – 320 nm) and UVA (320 – 400 nm) wavelengths and have been reported in green, red and brown algae from tropical, temperate and polar regions. Seven species collected in Espírito Santo State (Brazilian southeast coast) were selected for this study: *Laurencia aldingensis* (LA-U07, LA-U08, LA-C), *L. catarinensis* (LC), *L. dendroidea* (LD), *Laurencia* sp. 1 (LI-08, LI-11), *Laurencia* sp. 2 (LPV), *L. translucida* (LT) and *Palisada perforata* (PP). The MAAs were extracted with methanol 100% and analyzed by HPLC-MS. Shinorine (RT = 1,00 min; mass = 332 amu; λ_{max} = 332 nm), porphyra-334 (RT = 1,55 min; mass = 346 amu; λ_{max} = 332 nm), palythine (RT = 1,06 min; mass = 244 amu; λ_{max} = 320 nm) and the presumed asterina-330/micosporine-metilamine-serine (RT = 1,30 min; mass = 288 amu; λ_{max} = 325 – 330 nm) seem to the ubiquitous among these algae. One more detected peak was the cis-trans pair palythene/usujirene (mass = 284 amu; λ_{max} = 354 – 360 nm), present in LA.C, LC, LD, LPV and LT. Among the studied samples LC and LPV present the greater number of peaks (11), while LI presents the fewest (5). We could observe differences in terms of quantity and diversity of MAAs between samples of the same species collected at different dates suggesting that the environment contributes to the synthesis of these molecules. LT and LPV present peaks with m/z ratios never described in literature, suggesting that they may possess novel MAAs.

PI105 New alkaloids, Stemoxazolidinones A – F from Stemonae Radix

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Plants belonging to the genus *Stemona* (family Stemonaceae) are distributed in Southeast Asia, and are known to produce alkaloids of unique structures, which are characterized by incorporating a pyrrolo[1,2-a]azepine nucleus. In continuation of our phytochemical investigation of Stemonae Radix, we isolated six new alkaloids, stemoxazolidinones A – F (Although the crude drug has been previously considered to be *S. sessilifolia* (Miq.) Miq. from morphological features and the marketability of the locality, it was identified as *S. tuberose* Lour from our reinvestigation of its botanical identity due to its nucleotide sequences of chloroplast DNA *pet*B-*pet*D and *trn*K-*rps*16 regions being completely identical to those of *S. tuberosa* Lour. deposited in GenBank (Accession No. AB490134Tb1 and AB490140Td3)). The structures of stemoxazolidinnones A – C and E were determined by interpretation of their spectroscopic data and those of stemoxazolidinones D and F by X-ray chrystallography. These alkaloids possess a novel structural unit in which an oxazolidin-2-one unit fuses with a pyrrolo[1,2-a]azepine nucleus of the rearranged or normal tuberostemonine-type skeleton, namely, those two ring units are fuse together at positions 1 and 9a or 9 and 9a of the pyrrolo[1,2-a] azepine ring with the nitrogen of the oxazolidin-2-one linking to C-9a to construct a six ring system in the molecule. Such structural features have not been reported in the alkaloid skeleton before. Further, stemoxazolidinones A – C possess a novel carbon framework in their mother skeleton in which the C-1 of a tuberostemonine-type skeleton was rearranged from C-12 to C-11 to form a spiro structure.

PI106 GC-MS Analysis of the essential oil of *Alpinia zerumbet* (Pers.) B.L. and *in vitro* hepatoprotection and cytotoxicity study. MPC-4

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Alpinia zerumbet (Pers.) B.L. Burrt & R.M.Sm. Family Zingiberaceae is commonly known as shell ginger. It is used in folk medicine for its anti-inflammatory, bacteriostatic and fungistatic properties. It is also used for treatment of cardiovascular hypertension and as an antispasmodic agent. The preliminary phytochemical screening of rhizomes and leaves revealed the presence of volatile oil and flavonoids. The essential oil of the fresh leaves and rhizomes was obtained by hydro-distillation. GC-MS analysis revealed the presence of many compounds qualitatively. 1,8-cineole and terpinen-4-ol were identified as major compounds in the essential oil of leaves and rhizomes respectively. *In vitro* monolayer of rat hepatocytes revealed that the LC_{50} of the aqueous extract of dried rhizome powder was more than 1000 µg/ml and that of the total methanolic extract of dried rhizome was 500 – 750 µg/ml in concentration compared to the control cells. At the same time the hepatoprotective activity on monolayer hepatocytes for aqueous extract was100 µg/ml and for the methanolic extract was 25 – 50 µg/ml in concentration. On the other hand the in *in vitro* cytotoxicity study showed that the aqueous extract exerted toxicity on U937 cells at a concentration of 100 µg/ml while it was safe on PBMC cells. For the cytotoxicity of the methanolic extract, it was safe on both U937 and PBMC cells till 100 µg/ml in

concentration. **References:** [1] Egyptian Pharmacopoeia, English text, 3rd Ed., University Press, Cairo, (1984). [2] Finar, I. L.; "Organic Chemistry", 6th Ed., Longman group Ltd., England, 445 (1973). [3] Adams, R. P.; "Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy", Allured Publishing Corp., Carol Stream, Illinois, U.S.A., (1995).

PI107 Highly oxygenated sesquiterpenes from the aerial parts of *Artemisia alba* Turra

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Artemisia alba Turra (*Artemisia,* Asteraceae) is widespread in the southern and south-eastern parts of Europe – Spain, Italy and Balkan peninsula [1]. The aerial parts of *Artemisia alba* Turra have been traditionally utilized as a stomach digestive and tonic in the form of a decoction [2]. The literature survey revealed few reports concerning the non-volatile components of this species: santonin, nerolidol derivatives and artalbic acid were isolated from the aerial parts, whereas the roots were shown to contain a sesquiterpene-coumarin ether [3]. The aerial parts of field cultivated *Artemisia alba* Turra were initially defatted with hexane and then extracted successively with chloroform and methanol. Further CC and PTLC purification of the chloroform extract resulted in the isolation of 10 new compounds with germacrane, oplopane, eudesmane, guaiane and bisabolane carbon skeletons. Their structures were elucidated by NMR spectroscopy (^{1}H-NMR, ^{1}H-^{1}H COSY, HSQC, HMBC, NOESY) and MS. It was found that the isolated compounds are highly oxygenated and contained 2 – 5 oxygen-bearing functions (OH, OOH, and OAc). The main components were germacrane derivatives with C-10 exomethylene group and C-3 and C-9 substituted positions. Additional oxygenated positions were C-1, C-2, C-4 or C-12 carbons. The dominant component among the isolated compounds was 1-hydroperoxy-3-hydroxy-9-acetoxy-germacra-5, 10(14)-diene. Acknowledgments: Swiss National Science Foundation in the framework of the Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZ0_142989; DO2 – 1153) **References:** [1] Tutin T.G., et al. Artemisia L. In *Flora Europaea*; (Tutin, T.G., et al. Eds.), Cambridge Univ. Press: Cambridge, UK, V. 4, 178 – 186, 1976. [2] Rigat M. et al. *J. Ethnopharmacol., 113, 267 – 277, 2007.* [3] Maggio A. et al., *Tetrahedron Lett.*, **52**, 4543 – 4545, 2011 and literature cited therein.

PI108 Polar secondary metabolites from *Juglans regia* pericarps

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The aim of this study was to investigate the constituents of the acetone: H_2O (70: 30) extract of *Juglans regia* fresh pericarps, in continuation to our previous studies [1, 2]. The extract was fractionated by liquid – liquid extraction using ethyl acetate, n-butanol and water. The derived ethyl acetate extract was further fractionated through semi-prep RP_{18}-HPLC and afforded sclerone, 4-hydroxy-1-tetralone, regiolone, vanillic acid, 4-hydroxyphenylethyl 4-hydroxybenzoate, (7*S*, 8R)-dihydrodehydrodiconiferyl alcohol. The latter two compounds have been found for the first time in *Juglans regia* L. The structures of the isolated compounds were confirmed by spectroscopic methods, including 1D- & 2D-NMR, UV-Vis. In our previous study, it was found that sclerone and 3-MeO-juglone exhibit cytotoxic activity against a panel of cancer cell lines (MTT assay), since both compounds increased NK cell cytotoxicity as well as they enhanced T cell proliferation as assessed by ^{3}H-thymidine incorporation assays [2]. The investigation of the extract concerning its content and the *in vitro* anticancer and immunomodulating activities is still under progress. **References:** [1] Kavroulaki, E. et al. (2008) Planta Med. 74: 1040. [2] Tsasi et al. (2012) Planta Med. 78: 1196. **Acknowledgements:** This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

PI109 Antioxidant phenolic compounds from *Alchornea floribunda* leaves

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Alchornea floribunda leaves have been previously shown to posses potent anti-inflammatory compounds[1 – 3]. Further chemical investigation of a flavonoid sub-fraction of the methanol leaf extract of *Alchornea floribunda* led to the isolation of three flavans (-) catechin (+) catechin (-) epicatechin and a flavanone (2R, 3R) dihydroquercitin. The structures of these compounds were elucidated by 1 D and 2 D NMR spectroscopy and Liquid Chromatography- Electrospray Ionization Mass Spectrometry (LC-ESIMS). All the isolated compounds were assessed for their antioxidant potential *in vitro* using DPPH free radical scavenging and Ferric reducing ability[4]. In the DPPH model, (-) epicatechin, (+) epicatechin and (2R, 3R) dihydroquercitin showed high free radical scavenging activity with IC_{50} values of 19, 40 and 46 µg/mL respectively with the activity of (-) epicathechin comparable to that of the standard, chlorogenic acid (IC_{50} = 22 µg/mL). (-) catechin showed only mild activity with IC_{50} value of 88 µg/mL. In the Ferric reducing test, (-) catechin, (-) epicatechin and (+) epicatechin exhibited IC_{50} values of 46, 68 and 88 22 µg/mL respectively with the activity of (-) epicathechin higher than that of the standar, ascobic acid (IC_{50} = 66 µg/mL). (2R, 3R) dihydroquercitin showed poor Ferric reducing activity (IC_{50} > 100 µg/mL). The observed antioxidant activities of the isolated flavans may explain the efficacy of the extract of *Alchornea floribunda* leaves in the ethnomedicinal management of diseases associated with oxidative stress. **References:** [1] Okoye FBC, Osadebe PO (2009). *Asian Pacific Journal of Tropical Medicine*, 2(3): 7 – 14. [2] Okoye FBC, Osadebe PO, Proksch P, Edrada-Ebe RAl, Nworu CS, Esimone CO. (2010). *Planta Medica* 76 (2): 172 – 177. [3] Okoye FBC, Osadebe PO. (2010). *Natural Product Research* 24 (3): 266 – 273. [4] RACHH RR etal., (2009) ROM.J.Bio.Plant Biol., vol 54. No2. P. 141 – 148.

PI110 New flavanone glycoside from the leaves of *Faramea marambaiae* (Rubiaceae)

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The species *Faramea marambaiae* M. Gomes (Rubiaceae) is endemic in the city of Rio de Janeiro, Brazil, and there are no reports about its chemical composition or therapeutic potential. From the methanol extract of its leaves were isolated, among other compounds, by liquid-liquid partition followed by reverse phase (C18) column chromatography (CC) and semi-preparative reverse-phase (C18) high performance liquid chromatography coupled to a diode array detector (HPLC-DAD) the novel flavanone glycoside: 5-hydroxy-4'-methoxy-flavanone-7-*O*-ß-D-apiofuranosyl-(1 – 6)-ß-D-glucopyranoside. Structural determination was made by nuclear magnetic resonance (NMR) techniques in one and two dimensions (^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC), ultraviolet spectroscopy (UV) and optical rotation. Preliminary test for *in vitro* cytotoxicity of the methanol extract towards a human breast cancer tumor cell line (MCF-7) showed no cytotoxicity. Tests for antioxidant activity with DPPH and phototoxic activity (photosensitizer action to produce singlet oxygen – $^1O_2$) of the flavonoid rich methanol:water 9:1 fraction, obtained by partition of the methanol extract, showed moderate antioxidant activity (IC_{50}= 174.96 µg/mL) and absence of phototoxicity. Acknowledgments: CNPq and FAPERJ (Brazil), Oswaldo Cruz Foundation NMR Lab.

PI111 Chemical constituents from *Neonectria coronata* YMJ94043006

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Four novel 10-membered macrolides, namely neonectrilactones A–D (**1**–**4**), together with four cylindrols **5**–**8** and three ascochlorins **9**–**11**, were isolated from the ethyl acetate extracts of the fermented broths of *Neonectria coronata* YMJ94043006. The structures of **1**–**11** were elucidated on the basis of spectroscopic data analyses. The relative configurations of **1**–**4** were deduced mainly by ^{1}H-NMR and NOESY, and compared with the literatures. Compounds **5**-**11** were subjected to growth inhibitory activity against A549 lung cancer line, and among the compounds tested, the chlorine-containing cylindrol analogues **9**–**11** exhibited potent activities with GI_{50} values of 1.2, 1.1 and 8.2 μM, respectively, while **5**–**8** exhibited no activity even at a concentration higher than 30 μM. We thus suggested that the chlorine would play a crucial role for the cytotoxicity of **9**–**11**.

PI112 Five new components from the aerial part of *Lindera akoensis* with anti-inflammatory activity

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Lindera akoensis (Lauraceae) is an endemic evergreen tree that grows in broad-leaved forests in lowlands throughout Taiwan. Aporphines, alkaloids, sesquiterpenoids, butanolides, furanoids, chalconoids, and phenolic compounds were widely distributed in the plants of the genus *Lindera*. Some isolates exhibit biological activies, including anti-mycobacterial, anti-inflammatory, anti-human lung cancer (SBC-3), osteoclast differentiation inhibitory, human slowing down of the pregession of diabetic nephropathy in mice, anti-nociceptive, and LDL anti-oxidation effect *et al*.. In this study, we have isolated five new compounds, 3β-((*E*)-dodec-1-enyl)-4β-hydroxy-5β-methyldihydrofuran-2-one (**1**), 3α-((*E*)-dodec-1-enyl)-4β-hydroxy-5β-methyldihydrofuran-2-one (**2**), linderinol (**3**), 4'-*O*-methylkaempferol 3-*O*-α-L-(4''-*E*-*p*-coumaroyl)rhamoside (**4**), kaempferol 3-*O*-α-L-(4''-*Z*-*p*-coumaroyl)rhamoside (**5**) as well as six known compounds. These components show *in vitro* anti-inflammatory activity decrease the LPS-stimulated product of nitrite in RAW 264.7 cell with IC_{50} values of 1.4 – 67.9 μM. (Indomethacin was used as positive control with IC_{50} value of 182.9 μm).

PI113 Phytochemical constituents of *Monochoria vaginalis* var. *plantaginea* and their antioxidative and cytotoxic activities

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The aquatic plant *Monochoria vaginalis* var. *plantaginea* (Roxb.) Solms (Pontederiaceae) is a traditional herbal medicine, and widely distributed in Asian countries including Korea, China, Japan, India, and Malaysia. The whole plant is considered as a functional food and has also been used for treatment of dysentery, enteritis, acute tonsillitis, gum abscesses, erysipelas, boils. Juice of the leaves is taken for coughs and that of the root for stomach and liver complaints, asthma and toothache. In our continuing studies to find bioactive compounds from natural products, several structures were isolated from the whole plant of *Monochoria vaginalis* var. *plantaginea*. Among five fractions of the MeOH extract, the n-hexane fraction exhibited comparatively higher antioxidative and cytotoxic activities than the other fractions. The CH_2Cl_2 and BuOH fractions also showed strong antioxidative activity. Ten compounds were isolated from the n-hexane and CH_2Cl_2 fractions, and their structures were identified as cholest-4-en-3,6-dione, stigmast-4-ene-3,6-dione, methoxyanigorufone, (10Z)-1-(2,6-dihydroxyphenyl)octadec-10-en-1-one, 1-(4-methoxyphenyl)-7-phenyl-(6E)-6-hepten-3-one, (+)-dehydrovomifoliol, 4-hydroxyphenylacetonitrile, (6R,9S)-vomifoliol, (6S,9S)-vomifoliol, (3S,5R,6R,7E,9R)-5,6-epoxy-3,9-dihydroxy-7-megastigmene by comparison of their spectral data with literature values. Cytotoxic and antioxidative activities for solvent fractions and isolated compounds were evaluated using SRB (sulforhodamin B) assay against human tumor cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15), and ORAC (oxygen radical absorbance capacity) assay, respectively.

PI114 Chemical composition and antibacterial activities of algerian *Pinus halepensis* Mill and *Pinus maritima* essential oils

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The chemical composition of the essential oils obtained from the needles of the two species *Pinus maritima* harvested from two areas, Hadada (site 1) and Tonga forests (site 2) in the National Park of El Kala, (North East of Algeria) and *Pinus halepensis* Mill. collected from Mellah Lake forest (site 3) and Zaarouria forest (site 4) (Souk Ahras, internal Algeria). The yield is respectively 0.27%, 0.18%, 0.81% and 0.3%. The essential oils investigated by gas chromatography coupled with the masse spectrometry type schimadzu equiped with FID, GC-MS analysis revealed the presence of *Pinus maritima* oils from (site 1). The major ones are Alpha-pinene (40.31%), Beta-caryophyllene (28.54%), and caryophyllene oxide (13.42%). In (site 2), the major compounds are Beta-cryophyllene (35.99%), Alpha-pinene (20.63%), Alpha-bulnesene (15.93%). The oil of *Pinus halepensis* Mill of (site 3) is composed mainly of Alpha-humulene (31.89%), Alpha-pinene (24.41%), Delta3-carene (19.38%), while that of (site 4) consists primarily of Aromadendrène (32.3%), Alpha-pinene (21.79%), Alpha-terpinolene (9.78%). The biological activity of essential oils (crude and diluted) tested against Escherichia coli; Staphylococcus aureus and Pseudomonas aeruginosa have showed antibacterial activities. Keywords: Essential oils, *Pinus maritima*, *Pinus halepensis* Mill., antibacterial activity.

PI115 Occurrence of (R_S,R_C)- and (S_S,R_C)-marasmin in *Tulbaghia violacea* Harv.

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The cysteine sulphoxide marasmin is a natural sulphur-containing aroma precursor, occurring in several plant species, as well as in some members of the genus *Marasmius.* After disruption of a cell containing it, it is cleaved by a C-S-lyase. The generated sulfenic acids lead to the formation of the thiosulfinate marasmicin which is the main odourous compound of *Marasmius* spec. and some of these plants. Marasmicin has been drawn attention to for further investigation due to bioactivity against fungi and *Mycobacterium tuberculosis*. [1] Marasmin was firstly identified as precursor of the garlic odour in *Marasmius* spec. [2]. In the fungi it appears as glutamyl-dipeptide with (S)-configuration at the sulphur atom. 11 years later it was found in the fruits of the tree *Scorodocarpus borneensis* Becc., where it exists in form of the free sulphoxide in (R)-configuration [3]. In the following marasmin was detected in several plants of the *Amaryllidaceae* including *Allium*, *Ipheion*, *Leucocoryne* and *Tulbaghia* [4]. As in *S. borneensis* only the (R_S,R_c)-marasmin could be detected in all plant material investigated so far. Recent research on the South African plant *Tulbaghia violacea* Harv., known as 'society garlic', revealed the presence of both configurations of marasmin (Fig.), with (R_S,R_c)-marasmin being the major compound. This is an example of the rare appearance of both isomers of one cysteine sulphoxide in the same plant as well as the first report of (S_S,R_c)-marasmin within the plant kingdom. This leads to the conclusion that either the oxidizing enzyme is not very specific or there is more than one enzyme involved in the oxidation of cysteine derivatives in this plant. **References:** [1] Kusterer J, Fritsch RM, & Keusgen M, *J Agric Food Chem.* (2011) *59*(15):8289 – 97 [2] Gmelin R, Luxa H-H, Roth K, & Höfle G, *Phytochem.* (1976) *15*(11):1717 – 1721 [3] Kubota K, Hirayama H, & Sato Y, *Phytochem.* (1998) *49*(1):99 – 102 [4] Kubec R, Krejčová P, Mansur L, & García N, *J Agric Food Chem.* (2013) *61*(6):1335 – 42

J. Plant phenols: structures, analytics and activities

PJ1 New phenolic glycosides from Nigerian mistletoe (*Loranthus micranthus* Linn.) parasitizing on *Hevea brasiliensis*

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Two (**2**) new phenolic glycosides, linamarin gallate (**1**) and walsuraside B (**2**), together, with twelve (**12**) known polyphenols (**3 – 14**) were isolated from the leafy twigs of Nigerian mistletoe *Loranthus micranthus* (Linn.) parasitic on *Hevea brasiliensis*. Compound **1** was characterized as an unusual cyanogenic glycoside. All the compounds were isolated for the first time from mistletoe. The structures of the new compounds were unambiguously elucidated by a combination of 1D (^{1}H, ^{13}C), 2D (COSY, HSQC, HMBC) NMR and Mass Spectroscopy (HPLC/ESI-MS, HR/ESI-MS). All the isolated compounds exhibited strong antioxidant activity with IC_{50} values in the range of 23.8 – 50.1 µM and were found to be more active than the reference compound, chlorogenic acid (IC_{50}= 67.9 µM) in the DPPH assay.

PJ2 Flavonoids from the aerial parts of *Eryngium campestre* L. with antioxidant and anti-alzheimer activities

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Eryngium is the largest and arguably the most taxonomically complex genus in the family Apiaceae. The genus is represented by 317 taxa widespread throughout Central Asia, northern Africa, America, Central and Southeast Europe [1]. *Eryngium campestre* (Field eryngo) is a rare perennial restricted to dry grassy areas near the coast. It is native to Spain, France, Germany and Greece and other scattered localities in Europe, and is also found in Africa and Asia [2]. It has been used in European herbal medicine as an infusion to treat whooping cough, kidney and urinary tract inflammations [3]. Phytochemical investigation of the methanolic extract of *Eryngium campestre* L. aerial parts led to isolation of eleven known flavonol glycosides. Structures were elucidated by spectroscopic and chemical methods. The methanol extract of *E. campestre* and the isolated flavonols exhibited moderate to strong antioxidant activity in DPPH radical scavenging and reducing power assays. *Eryngium campestre* extract showed significant inhibition of the β-amyloid Aβ 42 (IC_{50}= 155.75 ± 7.43 ng/ml) without significant reduction in total Aβ (Aβ 40 + Aβ 42) levels in human H4 cell line, using sensitive sandwich enzyme linked immunosorbent assay (ELISA). The results showed no inhibition activity of the extract against COX-1 and COX-2 up to 400 ng/ml concentration. **References:** [1] Wörz A. On the distribution and relationships of the South-West Asian species of Eryngium L. (Apiaceae-Saniculoideae). Turk J Bot 2004; 28: 85 – 92. [2] Clapham AR, Tutin TG. Flora of the British Isles. 1st ed. Cambridge: Cambridge University Press 1952. [3] Gruenwald J, Brendler T, Jaenicke C. PDR for Herbal Medicines. 2nd ed. New Jersey: Medical Economics Company 2000; 729 – 33.

PJ3 Protection against oxidative stress by rosmarinic acid and its major metabolites in hepatic cells

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Background and aims Accumulation of reactive oxygen species causing cellular oxidative stress contributes strongly towards the induction and progression of liver and neurodegenerative diseases [1]. Hence therapeutic focus of recent researches has shifted to antioxidants, with keen interest on those of plant origin. *Ocimum canum Sims*, an aromatic Ghanaian kitchen spice, is a traditional antidiabetic [2]. Rosmarinic acid (RA), a key phenolic compound of this herb could account for this therapeutic effect [2]. Rosmarinic acid impeded oxidant-induced cellular damage in mice [1]. Hence, antioxidant potential of RA and its metabolites were assayed. **Materials and methods** Antioxidant effects of rosmarinic acid (RA) and its key metabolites – caffeic acid (CA), ferulic acid (FA), m-coumaric acid (CoA), 3,4-dihydroxyphenyllactic acid (DPLA) were evaluated in DPPH-radical scavenging assay. Hepatoprotection against oxidant damage induced by 0.5mM *tert*-butyl hydroperoxide was assayed in HepG2 cells (20 hours pre-exposure and 5 hours co-exposure with chemicals). Quercetin (Q) and DMSO were positive and vehicle controls respectively. **Results**

Tab. 1: Comparing the antioxidant potential of phytochemicals in DPPH scavenging assay and cytoprotection assays.

Phytochemical	EC_{50} (µg/ml) ± SEM		
	DPPH	5hr co-exposure	20hr pre-exposure
Quercetin	62.2 ± 2.0	9.2 ± 1.0	29.0 ± 3.10
Rosmarinic acid	80.1 ± 7.6	249.2 ± 12.0$^{\alpha}$	285.0 ± 62.0$^{\alpha}$
Caffeic acid	43.4 ± 0.9$^{*\#}$	103.1 ± 10.7$^{\alpha\beta}$	60.1 ± 46.6$^{\alpha\beta}$
3,4-(dihydroxyphenyl)lactic acid	43.6 ± 0.7$^{*\#}$	No effect (> 400 µg/ml)	No effect (> 400 µg/ml)
Ferulic acid	94.7 ± 8.4$^{*\#\infty}$	No effect (> 400 µg/ml)	No effect (> 400 µg/ml)
m-Coumaric acid	No effect (> 320 µg/ml)	No effect (> 400 µg/ml)	No effect (> 400 µg/ml)

Antioxidant potencies of phytochemicals in chemical and cellular assays. Values are mean EC_{50}± SEM, n = at least 4 independent assays. *P (< 0.05) when compared to positive control. $^{\#}$P (< 0.0001) compared with rosmarinic acid, $^{\infty}$P < 0.001 compared with caffeic acid, $^{\alpha}$P < 0.0001 compared with quercetin and $^{\beta}$P < 0.0001 when compared with rosmarinic acid.

Conclusions: CA and DPLA are stronger DPPH-scavengers than the other chemicals; except CoA, having no antiradical activity. With no toxicities, Q protected more effectively than CA and RA in 5hr and 20hr pre-exposure protocols, in decreasing order. FA, CoA and DPLA were ineffective. Despite common theoretical basis, disparity between DPPH and 5hr co-exposure data indicates limitations in cytoprotection. Hence CA is a stronger antioxidant than RA, the parent compound. **References:** [1] Lima, C.F., et al., *Water and methanolic extracts of Salvia officinalis protect HepG2 cells from t-BHP induced oxidative damage*. Chemico-Biological Interactions, 2007. **167**(2): p. 107 – 115. [2] Berhow, M.A., A.O. Affum, and B.A. Gyan, *Rosmarinic Acid Content in Antidiabetic Aqueous Extract of Ocimum canum Sims Grown in Ghana*. Journal of Medicinal Food, 2012. **15**(7): p. 611 – 620.

PJ4 Rosmarinic acid content in antidiabetic aqueous extract of *Ocimum canum* Sims grown in Ghana

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Rosmarinic acid (RA) is an important antioxidant polyphenol that is found in a variety of spices and herbs, including *Ocimum canum* Sims (locally called eme or akokobesa in Ghana). Aqueous extracts from the leaves of *O. canum* are used as an antidiabetic herbal medicine in Ghana. Analytical thin-layer chromatography was used to examine the composition of the polyphenols in leaf extracts. The polyphenol content in the aqueous and methanol extracts from the leaf, as determined by the Folin-Ciocalteu method, were 314 and 315 mg gallic acid equivalent/g leaf sample, respectively. The total flavonoid concentration as determined by the aluminum (III) chloride method was 135 mg catechin equivalent/g leaf sample. High-performance liquid chromatography coupled to an electrospray Quadrupole time-of-flight mass spectrometer was also used to determine the polyphenol fingerprint profile in the leaf extracts of *O. canum*. Although the average RA concentration in the *O. canum* leaf extracts from Ghana was 1.69 mg/g dry weight (reported values range from 0.01 to 99.62 mg/g dry weight), it was still the prominent peak besides caffeic acid derivatives in the polyphenolic profile.

PJ5 Flavonoids from the leaves of *Physalis angulata* Linn

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Plants from the genus Physalis are particularly rich in secondary metabolites. *Physalis angulata* is a widely distributed annual plant belonging to the nightshade family Solanaceae. *Physalis angulata* have been used for centuries as medicinal herbs in the treatment of urinary tract infection, skin diseases, gonnorhea, ulcers, sores and as vermicidal drug [1,2]. The main purpose of this present study is to screen the leaves of this plant for phytochemical constituents that may be responsible for some of these biological activities. The dried leaves of *Physalis angulata* were sequentially extracted with dichloromethane and 70% methanol. The aqueous methanol extract was partitioned between ethyl acetate and n-butanol. From the n-butanol fraction, four Flavonoids were isolated through column chromatography over silica gel G and sephadex LH-20. Based on the spectroscopic data which include NMR and mass spectroscopy, the chemical structures were determined as quercetin (1), quercetin 3-O-methyl ether (2) isoquercetrin (3) and Kaempferol 7-O-rhamnoside (4). The structures were elucidated using spectroscopic technique and compared with literature [3 – 4]. Anti-oxidant activity using DPPH and anti-bacterial activity of the flavonoids were also investigated. These compounds were isolated for the first time from the leaves of this plant. Key words:*Physalis angulata*, flavonoids, quercetin, quercetin methyl ether, isoquercetrin and Kaempferol -7-O-rhamnoside **References:** [1] Choi, EM and Hwang MT. Journ of Ethnopharmacol 89,2003: 171 – 175 [2] Ismail, N and Alam, M. Fitoterapia 72,2001:676 – 679 [3] Mabry, TJ; Markham, KR and Thomas, MB. The Systematic Identification of Flavonoids, 1970,65 – 73,126 – 134 [4] Agrawal, PK. Carbon-13 NMR of Flavonoids, 1983. Elsevier Publication New York,162 – 184

PJ6 Proteinase-inhibiting activity of an extract of *Rumex acetosa* L. against virulence factors of *Porphyromonas gingivalis*

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Periodontitis is a disease with considerable impact on the whole organism and worldwide prevalence. The gram-negative bacterium *Porphyromonas gingivalis* is regarded as one of its main etiological agents. Essential for the progression of this disease are highly specialized virulence factors of *P. gingivalis*, Lys-gingipain (Kgp) and Arg-gingipain (Rgp). Both proteinases are involved in the process of adhesion to host cells, in the bacterial invasion into the cells, acquisition of nutrients and the modulation of the host's local immune response. In the context of developing oral hygiene products with antiadhesive activity against *P. gingivalis* a proanthocyanidin-rich acetone-water extract (7:3) of *Rumex acetosa* L. (Polygonaceae) [1] proved to be highly effective under *in vitro* conditions. Treatment of *P. gingivalis* with the extract led to concentration dependant gingipain inhibition. Especially Rgp activity was inhibited, by approximately 20, 60 and 80% at extract concentrations of 5, 10 and 50 µg/mL. For pinpointing active principles, dimeric and trimeric proanthocyanidins were isolated and their structural features characterized. Their influence on Kgp and Rgp activity was determined. While procyanidin B2 had no significant effect, epicatechin-3-O-gallate-(4b→8)-epicatechin-3-O-gallate showed about 80% inhibition of Rgp at a concentration of 5 µM. An isolated A-type procyanidin, epicatechin-(2b→7, 4b→8)- epicatechin-(4b→8)-epicatechin, inhibited Rgp selectively, without influencing Kgp. Therefore unspecific tannin-like astringent effects as reason for gingipain inhibition can be excluded with the utmost probability. Corresponding structure-activity relations can be used for development of novel inhibitors of bacterial gingipains for oral application. **Reference:** [1] Bicker A, Petereit F, Hensel A (2009) Proanthocyanidins and a phloroglucinol derivative from *Rumex acetosa* L. Fitoterapia. 80(8); 483 – 95.

PJ7 Cinnamaldehyde, carvacrol and selected organic acids affect expression of immune related genes in IPEC-J2 cells exposed to *Salmonella enterica* serotype Typhimurium or *Escherichia coli* K88

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In animal feeds, concentrations of phytochemicals and organic acids are probably not lethal to pathogens. However, previous studies have shown that sub-lethal concentrations of cinnamaldehyde, carvacrol and certain acids inhibit attachment and invasion of bacterial pathogens into IPEC-J2 cells. The aim of this study was to investigate the effects of cinnamaldehyde and organic acids on selected immune related genes in porcine jejunum epithelial (IPEC-J2) cells when exposed to *S.* Typhimurium or *E. coli* K88 to find out whether such effects could contribute to the previously observed reduction of virulence. IPEC-J2 cells were grown to confluence and exposed to *E. coli* O149:K91:K88 strain 498 or *S.* Typhimurium ATCC 14028 for 1 h in the presence and absence of cinnamaldehyde, cinnamic, lactic or propionic acid. qRT-PCR analysis was carried out using primers for immune related genes: IkBa (a marker for the crucial inflammatory mediator NFkB), Heat shock proteins Hsp70, Hsp70.2, Hsp27, hypoxia inducible factor HIF-1a and Nrf2. Expression levels were normalized to untreated control cells. Results showed fold changes in gene expression levels in the presence/absence of phytochemicals and/or organic acids and after bacterial exposure. (e.g. Figure 1 shows expression levels of IkBa). Bacterial attack induces IL-8 and IkBa but in the presence of cinnamaldehyde or carvacrol in combination with food acids adherence/invasion is controlled and this is reflected in the expression levels. Cinnamaldehyde induces Hsp70 and IL-8 indicating cell stress, but Hsp-70 may protect cells if induced before contact with bacteria. Unchanged expression levels of IL-12 indicate that these compounds do not act as anti-inflammatory agents, which is confirmed by the unchanged expression of NOD 2, which mediates anti-inflammatory signals.

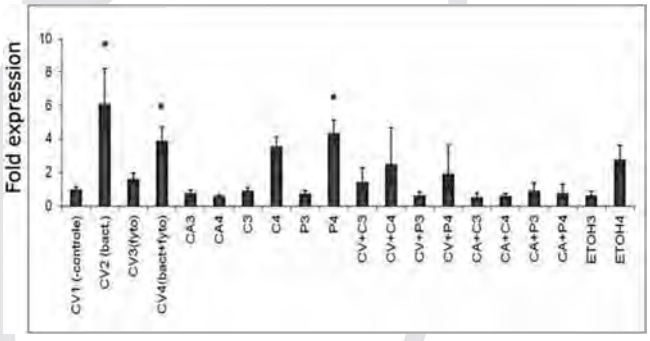

Fig. 1: Expression levels of IkBa in IPEC-J2 cells in presence/absence of phytochemicals and/or organic acids and after bacterial exposure.

PJ8 Activity-guided isolation of antioxidant principles from the Algerian desert herb *Fagonia* sp. (Zygophyllaceae)

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There is great interest in finding antioxidants from natural sources, which could be used in medicine and additive to nutraceuticals to prevent such deleterious effect and to minimize oxidative damage to cells. As a part of our investigation into medicinal plants growing in Algerian Sahara; in this study we investigate for the first time the antioxydant activity and compare phenolic and flavonoids contents of extracts from the aerial part of *Fagonia sp.(F. glutinosa & F. longispina)* used as medicinal plants by local population in Algerian Sahara. Hexane, Chloroform, Methanol and Ethyl acetate extracts from these deserts herb were tested

in vitro for their antioxidant activity determined by DPPH radical scavenging. The total phenolics and flavonoids content of the extracts were determined, in order to establish the correlations between these contents and the measured activities. Methanol and Ethyl acetate extracts exhibited a strong antioxidant activity which may be caused by the presence of Phenolic compounds. Thus from the ethyl acetate extract of the aerial parts of *F. Glutinosa* six flavonoids derivatives were characterized as: isorhamnetin 3-glucoside, kaempferol 7-*O*-glucoside, kaempferol 3-*O*-glucoside, kaempferol-7-*O*-rhamnoside, acacetin-7-*O*-rhamnoside and 8-methoxyflavones which seem to be the major antioxidant components of the bioactive fractions.

PJ9 Antioxidant potential of *Cymbopogon citratus* (lemongrass) polyphenols

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Oxidative stress is related to the physiopathology of many diseases[1] and takes place when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, which can generate important cell damage. Polyphenols and, specially, tannins have been described as powerful radical scavengers which may be very useful in alleviating oxidative stress and inflammation-related pathologies[2]. In this work the potential of lemongrass (*Cymbopogon citratus* (DC) Stapf.) phenolic compounds has been evaluated. Its infusion (CCI) and tannin-rich fraction (CCT) antioxidant activity has been tested by *in vitro* assays (DPPH, FRAP and TEAC). CCI and CCT were obtained as previously described by Figueirinha (2010). Chemical composition of both samples was attained by HPLC-PDA. Antiradical capacity was assessed by DPPH assay, described by Blois[3]. Ferric reducing ability (FRAP) was evaluated according to Benzie and Strain[4]. Trolox-equivalent antioxidant capacity (TEAC) method, as described by Re[5], was used to determine the ABTS radical scavenging ability. Both samples have showed high antioxidant values (Table 1), CCT exhibiting a pronounced activity improvement, when compared to CCI. This data indicates that the antioxidant effect is correlated with the compounds present in the polyphenolic fraction and it corroborates the use of lemongrass in folk medicine.

Tab. 1:

Sample	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	TEAC VALUE* (mg/mL)	
			ABTS	FRAP
CCA	0.87	1.05	1.20	2.01
CCT	0.43	0.54	0.28	1.05

*TEAC = concentration of sample that possesses the same antioxidant capacity of Trolox® 1mM.

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PJ10 *Cymbopogon citratus* industrial waste as source of an anti-inflammatory drug

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Cymbopogon citratus (Cc) (lemongrass) essential oil has a high economic value due to its use in pharmaceutical, cosmetics and agriculture industries[1]. After essential oil extraction by hydrodistillation, the aqueous extract (HD) is discarded. Since Cc aqueous extracts have been used in folk medicine to treat inflammation-based pathologies[1], the therapeutic value of HD was hypothesized. Therefore, we aimed to evaluate the potential benefits of HD recovery by investigating its phytoconstituents, the anti-inflammatory properties, as well as the hepatotoxicity. Dry aerial parts of Cc were extracted by hydrodistillation. Chemical characterization, performed by HPLC-MS^n as previously[2], demonstrated a high content of phenolic compounds such as phenolic acids, *p*-coumaric acid being the most abundant, and flavonoids, namely luteolin and apigenin glycosides. HD anti-inflammatory properties were evaluated in LPS-stimulated Raw 264.7 murine macrophages by measurement of nitric oxide (NO) production using Griess assay, as previously[3]. HD strongly decreased NO production, which supports the anti-inflammatory activity and puts in evidence the therapeutic potential of this aqueous extract. Considering a possible oral administration *in vivo*, the cell viability of HepG2 human hepatocytes was evaluated by MTT assay. HD did not showed hepatotoxicity, which indicates the safety of this extract. In conclusion, the present work discloses the HD phytochemistry, reveals its anti-inflammatory potential and evidenced HD hepatic safety. **Acknowledgements:** FCT for the projects PTDC/SAU-FCF/105429/2008, FEDER/COMPETE (FCOMP-01 – 0124-FEDER-011096) and PEst-OE/SAU/UI0177/2011, and Laboratory of Mass Spectrometry of CEF/UC node, integrated in the National Mass Spectrometry Network (RNEM) of Portugal, for the mass spectra. **References:** [1] Negrelle and Gomes. *Rev Bras Pl Med,* 2007, 9: 80 – 92 [2] Figueirinha, *et al. Food Chem*, 2008, 110: 718 – 28 [3] Francisco, *et al. J Ethnopharmacol*, 2011, 133: 818 – 27

PJ11 Effect of oleacein, polyphenol of olive oil, with natriuretic peptides on the functions of human neutrophils

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Polyphenols of extra virgin olive oil, such as oleacein (3,4-DHPEA-EDA), are believed to play a vital role in the prevention of cardiovascular diseases. It is considered that natriuretic peptides, which amount increase in acute myocardial infarction, exert protective effects against neutrophils- induced endothelial injury. The inhibitory effect of natriuretic peptides, such as atrial (ANP) and brain (BNP), is partly associated with the suppression of functions of neutrophils [1]. Taking into account that NEP is responsible for the degradation of natriuretic peptides, our aim was to establish the effect of oleacein on NEP activity in neutrophils. Additional purpose was to determine the influence of oleacein with or without ANP and BNP (10 ng/mL) on the selected functions of human neutrophils, such as elastase, metalloproteinase 9 (MMP-9), interleukin (IL-8) release from neutrophils. The determination of NEP activity was performed with spectrofluorimetric method using Suc-L-Ala-L-Ala-Phe-7-amino-3-methyl-coumarin (SAAP-AMC) as a substrate. The inhibition of elastase release were determined using colorimetric method. The effect on MMP-9 and IL-8 production was measured by ELISA assays. Oleacein inhibited NEP activity (IC_{50}= 43.2 ± 4.0 μM). Oleacein in the concentration of 20 μM reduced elastase release by 11.0 ± 5.3% (vs. 38.3 ± 5.1% with ANP; 25.4 ± 5.0% with BNP), IL-8 production by 12.9 ± 3.3% (vs. 25.7 ± 2.5% with ANP) and almost did not reduced MMP-9 production (vs. 14.3 ± 4.4% with ANP; 13.5 ± 4.1% with BNP). Unexpectedly oleacein with BNP increased IL-8 production. Oleacein by inhibiting NEP activity may enhance the protective effects of natriuretic peptides on the endothelium, what may partly explain the role of olive oil in the prevention of cardiovascular diseases. **Reference:** [1] Matsumura, T. et al.; J. Clin. Invest. (1996), 97: 2192 – 2203

PJ12 Phytochemical study on phenolic compounds of some species with anti-inflammatory potential of wild plant population in the North-Eastern Carpathians from Romania

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The mountain area is a valuable source of medicinal plant species, phytochemically not enough studied, in the context of environmental im-

pact. In the Romanian Eastern Carpathians their sustainable use – by the development of local the entrepreneurship is still a want. The aim of this study is to evaluate the phenolic content of some well-known species with a series of specific compounds: *Arnica montana* (sequiterpene lactones), *Veronica officinalis* (iridoids) and *Vaccinium vitis-idaea* (arbutin, proanthocyanidin), compounds having an antiinflammatory potential. The plant material was collected from the wild populations in the Bistrita Valley. The qualitative analysis of methanolic extract was performed by TLC and RP-HPLC-UV and the quantitative one was made by VIS spectrophotometry. In *A. montana* vegetative organs were analyzed, the highest content in phenolic acids and derivatives was found in leaves and basal rosettes: 157.33 – 227.58 mg/100 g d.w. (chlorogenic acid equiv.). The flavonoid apigenin was found in amounts of 3.33 – 10.83 mg/100 g d. w. in leaves and basal rosettes. In *V. officinalis* herba, depending on the source, the content of phenolic acids varied between 21.34 – 58.24 mg/100 g d.w. (chlorogenic acid equiv.), and that of flavonoids between 1.15 – 3.11 mg/100 g d.w. (luteolin equiv.). In the dried fruits of *V. vitis-idaea,* the content of phenolic acids was ranging from 554.35 – 569.87 mg/100 g d.w. (caffeic acid equiv.), while the phenolic content was of 3.788 – 4.048 g/100 g d.w. (gallic acid equiv.). One may conclude that by the content of the analyzed polyphenols (known for their antioxidant activity) in addition to the specific compounds, the mentioned species constitute some of the most important resources of bioactive compounds with antiinflammatory potential [1, 2, 3]. **References:** [1] Gründemann C *et al.*, J Ethnopharmacol. 2013, 145(1):118 – 26. [2] Mane C *et al.*, J. Agric. Food Chem. 2011, 59:3330 – 9. [3] Zheleva-Dimitrova D *et al.*, Turk J Biol, 2012, 36:732 – 7.

PJ13 Studies on antioxidant potential and active compounds of *Scutellaria salviifolia* Bentham

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The genus *Scutellaria* (Lamiaceae), includes about 350 species, is widespread in all over the world[1]. There are 15 *Scutellaria* L. species in Turkish flora and 3 of which are endemic[2]. Infusions of most of the species are used as tonic, antidiarrheaic, hemostatic, for wound healing, in gastric disorders, nausea and allergies[3, 4]. *Scutellaria* species and their active principles possess antitumor, anti-angiogenesis, hepatoprotective, antioxidant, anticonvulsant, antibacterial, and antiviral activities. Investigations on *Scutellaria* were resulted isolation of flavonoids, iridoid glucosides, phenylethanoid glycosides, diterpenes, triterpenoids, alkaloids and essential oils[1]. In the present study, aqueous extract of endemic *S. salviifolia*, collected from Ankara, Turkey; was tested for its radical scavenging activities against DPPH, SO, NO and ABTS radicals spectroscopically[2]. The extract showed concentration dependent radical scavenging ability. Its gallic acid equivalent total phenolic content was found as 72,3 mg/g dry extract using Folin-Ciocalteau reagent. On the other hand, the aqueous extract of the endemic *S. salviifolia* was subjected to polyamide column chromatography for the fractionation. Eluted fractions were tested for their radical scavenging activities. The most active phenylethanoid-rich fraction eluted with %50 MeOH from polyamide column was applied to HPLC-DAD system. The comparison of this fraction with previously isolated phenylethanoid glycosides showed the presence of acteoside, leucosceptoside A, martynoside and teucrioside as major phenylethanoid glycosides. Isolation studies have still been continuing on the titled plant. **References:** [1] Shang, X. et al. (2010) J Ethnopharmacol, 128, 279. [2] Davis, P.H. (1982). Flora of Turkey and East Aegean Islands. Vol. 7. University Press. Edinburgh. [3] Baytop, T. (1999). Therapy with Medicinal Plants in Turkey. İstanbul: Nobel Tıp Kitabevleri Ltd. [4] Patel, S.P. et al. (2013) Current Cancer Therapy Reviews, 9, 34.

PJ14 Chemical Compositions and Biological Activities of *Hypericum neurocalycinum* an Endemic Species of Turkey

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The genus *Hypericum* (Hypericaceae) has nearly 100 taxa grouped under 19 sections in Turkey. Among them, 45 taxa are endemic. In this study, chemical composition and biological activities of flowering aerial parts of the *H. neurocalycinum* an endemic species of Turkey belonging to Taeniocarpium section were determined and compared to *H. perforatum*. Quantitative determination of 11 major active components were performed by HPLC. The total phenolic and total flavonoids contents, antioxidant, cytotoxic and antimicrobial activities of the methanolic extracts of *H. neurocalycinum* (HNM) and *H. perforatum* (HPM) were investigated. The chemical compositions of the extracts are presented in Table 1. The total phenolic compounds of HNM and HPM were found as 23.11 ± 1.5 mg/g; 34.65 ± 0.9 mg/g and total flavonoid content of HNM and HPM were found as 22.80 ± 1.8 mg/g and 31.80 ± 0.9 mg/g of dry weight, respectively. The antioxidant activity results showed that the extracts have high antioxidant potential (2.74 mM ≥ EC_{50} values ≥ 0.288 mM). There was a high correlation between total phenolic, flavonoid contents and antioxidant activity. According to MTT results, the extracts demonstrated very low toxicity at high concentrations against HeLa and NRK-52E cell lines (EC_{50} values ≥ 0.541 mg/ml). Concerning the antimicrobial activity results, HNM and HPM have shown the highest activity against *Staphylococcus aureus*, the methicillin resistant *S. aureus* (MRSA), *S. epidermidis*. The extracts have no activity against *Candida albicans*.

Tab. 1: % Value of the chemical contents of *H. neurocalycinum* and *H. perforatum*

Compounds	*H. neurocalycinum* (%)	*H. perforatum* (%)
Pseudohypericin	0.00016	0.07475
Hypericin	0.00322	0.06729
Hyperforin	-	3.37501
Chlorogenic acid	0.26033	0.00802
Rutin	0.03299	1.01533
Hyperoside	0.30434	0.49113
Isoquercetin	0.12173	0.38795
Quercitrin	0.00482	0.00181
Kaempferol	-	0.13489
Quercetin	0.13809	0.12503
Amentoflavone	0.00144	0.00582

PJ15 Study of the cyclooxygenase inhibition by polyphenol extracts derived from monocultivar oils from autochtonous Maltese olive tree varieties

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The Maltese Islands form a small archipelago in the Mediterranean Sea that have been isolated physically from the closest European and African continents since the late Miocene period when harsh tectonic movements resulted in the small landmass. During the ensuing temporal and geographical isolation, flora and fauna have been subjected to intense selection pressure resulting in diversification from the forms found on the mainlands. In this poster, the results of a study on the anti-inflammatory activity of phenolic products from monocultivar oil extracts derived from two indigenous olive drupe varietals are presented. Polar extracts were isolated from the oil using methanol-water and subsequently dried using rotary evaporation and freeze drying. The total antioxidant activity in the polar polyphenol fractions as well as suitable controls was determined using the Folin-Ciocalteu reagent (Sultana *et al.*, 2009) followed by GC analysis. Extracts at test concentrations

of 2 ppm and 200 ppm were tested on lipopolysaccharide (LPS) (1 microgram/mL) stimulated human macrophage cells. Cyclooxygenase-2 (COX-2) inhibition was studied using the Cayman enzyme immunoassay-chemical kit.

PJ16 Evaluation of cytotoxic activity and active compounds from genus *Digitalis*

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In the Flora of Turkey, the genus *Digitalis* is represented by 9 species (1). Since most of the researches are focused on the cardenolides, other active compounds such as phenolics haven't been investigated for their role in biological activity of *Digitalis* extracts (2). In this study, comparative biological investigations were performed on the aqueous extracts of *D. ferruginea* L. subsp. *schischkinii* (Ivan) Werner and *D. lamarckii* Ivan on the basis of their cytotoxic activity. Cytotoxic activity was determined through the Hep-2 cancer cell line by MTT method (3). It was found that both extracts showed concentration dependent cytotoxicity. The extract of *D. ferruginea* subsp. *schischkinii* has shown 60,09% inhibition, while the inhibition of *D. lamarckii* is 35,13%, at 200 μg/ml concentration. Since the extract of *D. ferruginea* subsp. *schischkinii* was determined to be more active, it was applied to polyamide column chromatography for the bioactivity guided fractionation. The main fractions of the polyamide column were also investigated for their cytotoxic activity against Hep-2 cell line. Fraction, rich in phenylethanoid glycosides, was found as the most active which showed 52% inhibition at 200 μg/ml. Phytochemical studies on the active fraction were resulted in the isolation of seven compounds. Isolated compounds also showed cytotoxic activities against Hep-2 cell line between the IC_{50}:100 – 150 mg/ml. Structures of the isolated three compounds were determined as forsythiaside, calceorioside A, and methyl caffeoate by extensive NMR techniques. Structure determination studies on the other bioactive compounds have still been continuing. **Acknowledgement:**This study was supported by TUBITAK Project No:108T518. **References:** [1] Davis PH (1978) Flora of Turkey and East Eagean Islands, University Press, Edinburg, Vol 6. [2] Calıs I, et al.(1999) Pharmazie, 12,1926 – 1930 [3] Saracoglu I, et al.(1995) Biol Pharm Bull,18,1396 – 1400

PJ17 New acetylated flavonol glucuronides isolated from Polygoni avicularis herba and their influence on some neutrophiles' functions

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Polygoni avicularis herba is a pharmacopoeial medical material used for upper respiratory tract disorders and as diuretic agent. It contains flavonoids as a dominating group of compounds. Previous studies have shown that besides some well-known compounds such as avicularin, isoquercitrin and hyperoside, extracts from Polygoni avicularis herba contain significant quantities of several flavonol glucuronides which presence have not been confirmed before in this plant material (*1*). The extract for isolation of chosen compounds was prepared by heating dry plant material with 70% methanol on water bath. After evaporation of methanol extract was partionated between $CHCl_3$, AcOEt and n-butanol to obtain fraction of different polarity. Main constituents were isolated by column chromatography on Diaion HP-20 and Toyopaerl HW-40F followed by preparative HPLC. 10 flavonol glucuronides were isolated and their structure was determined by 1D and 2D NMR experiments. Compounds P7, P8 and P9 were isolated for the first time form any plant material. The presence of the rest of compounds was confirmed for the first time in Polygoni avicularis herba. The bioactivity of isolated compounds were evaluated in human neutrophil model. All the compounds were able to scavenge reactive oxygen species in f-MLP and PMA stimulated neutrophils. Isolated constituents were also able to inhibit the elastase release from stimulated neutrophils. The bioactivity of investigated compounds was correlated with differences in their chemical structure. **Reference:** [1] Granica, S.; Piwowarski J. P.; Popławska M.; Jakubowska M.; Borzym J.; Kiss A. K. Novel insight into qualitative standardization of *Polygoni avicularis herba* (Ph. Eur.). *Journal of Pharmaceutical and Biomedical Analysis* **2013**, *72*, 216 – 222.

PJ18 Antioxidant and cytotoxic activities of *Vitex agnus-castus* from five different regions of Turkey

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Vitex agnus-castus (the chaste tree) is a shrub of the Verbenaceae family growing in the Mediterranean region and Central Asia. It has been used for the treatment of menstrual problems including premenstrual symptoms and spasmodic dysmenorrhea. Traditionally, the fruits of the plant have been used as a digestive, stomachic, diuretic, antifungal and antianxiety in Turkey. The aim of this study was to investigate the total phenolic content as well as the antioxidant and cytotoxic activities of the methanolic extracts of the fruits of *V. agnus-castus* collected from five different regions of Turkey, namely Manavgat, Bodrum, Altınoluk, Enez, Zonguldak. The antioxidant capacities of the extracts were evaluated with DPPH radical-scavenging activity assay, including total phenolic content. The cytotoxic potential of the extracts was determined by MTT and LDH assays on HeLa-cells (human cancer cell line) and NRK-52E-cells (rat kidney cell line). In our previous study, the casticin amount of these extracts was determined by HPLC. Results revealed that casticin has been found in high amount in Manavgat and Bodrum samples as the responsible antioxidant agents. Two extracts prepared from samples from Manavgat and Bodrum exhibited significant radical scavenging activity with higher total phenolic contents compared to the other extracts.

PJ19 Antioxidant and antimicrobial activities, and phenolic compounds of *Inula thapsoides* ssp. *thapsoides*

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The members of *Inula* genus are widespread in the world and are used for many disorders by different cultures. *Inula* species contain terpenic compounds, especially sesquiterpen lactones, flavonoids, glycolipids and anthranilic acid derivatives (1). *I. thapsoides* (Bieb. ex Willd.) Sprengel which is a rhizomatous perennial herb (45 – 150 cm), has two subspecies in Turkey. In this study, *I. thapsoides* ssp. *thapsoides* was investigated for its total phenolic content, antioxidant, antimicrobial potential, and phenolic compound profile (2). Antioxidant activities of water, methanol and ethyl acetate extracts of flowers, leaves and roots of the plant were evaluated by DPPH and ABTS methods. All the extracts showed antioxidant activity in different concentrations. Water extract of leaves and methanol extract of roots expressed strong antioxidant activities with both methods compared to other extracts. Antimicrobial activities of the methanol extracts of flowers, leaves and roots of the plant were determined against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *C. albicans* and *C. tropicalis* by agar dilution method. All extracts exhibited antibacterial and anticandidal activities with different MIC values ranging from 50 to 200 μg/ml. Root extract was found very active against the investigated microorganisms. As the liable antioxidant and antimicrobial compounds, chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempferol were analyzed qualitatively and quantitatively in the plant parts by RP-HPLC. While chlorogenic and caffeic acids were found in all the investigated extracts, myricetin was not determined in the plant. Consequently, investigated phenolics could be responsible for the potent antioxidant and antimicrobial activities of the plant. **References:** [1] Zhao, Y-M. et al. (2006) *Chem. Biodivers.*, 3:371 – 384. [2] Davis, P.H. (1982) Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh.

PJ20 Phenolic Compounds from the Flower of *Stewartia pseudo-camellia* and Their Inhibitory Effects on the Release of β-Hexosaminidase in RBL-2H3 Cells

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Stewartia pseudo-camellia Maxim. (=*S. koreana* Nakai) is a deciduous tree in the family Theaceae which is distributed in southern Korea [1]. Its fruit or bark of stem and root have been used as a folk medicine for the treatment of circulatory disorders, paralysis of the limbs, legs and arms, and several pains. Previous studies on *S. pseudo-camellia*, have investigated several biological activities including angiogenesis [2], anti-inflammation [3, 4], prevention and treatment of osteoporosis [5], and anti-oxidative effect [6]. The flower of *S. pseudo-camellia* was consecutively extracted with hexane and MeOH. The anti-allergic activity of MeOH extract was comparatively higher than that of second-generation H_1-antihistamine ketotifen as positive control. A new compound together with nine known compounds, (+)-catechin, quercetin-3-rhamnoside, methyl gallate, (-)-epicatechin, afzelin, aromadendrin, myricetin, kaempferol-7-O-rhamnosyl-3-O-rutinoside, and kaempferol-7-O-(4""-O-acetylrhamnosyl)-3-O-rutinoside were isolated from the MeOH extract. The structure of a new phenolic compound, named pseudocamellianin A, was determined as kaempferol-7-O-(4""-O-acetylrhamnosyl)-3-O-(4"'-O-*p*-coumaroyl)rutinoside from its spectral data. The anti-allergic activity of isolated compounds was evaluated in an *in vitro* assay on β-hexosaminidase release inhibition using RBL-2H3 cells stimulated by DNP-BSA. Among tested compounds, quercetin-3-rhamnoside, afzelin, aromadendrin, myricetin exhibited similar activity with ketotifen.

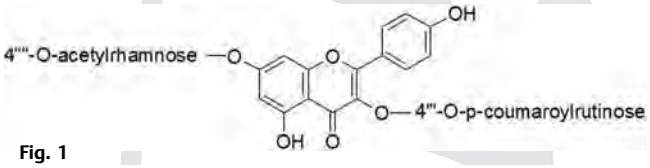

Fig. 1

References: [1] Lee, TB. (1999) Illustrated flora of Korea. Hyangmoonsa. Seoul. [2] Lee, TH et al. (2010) Phytother. Res. 24: 20 – 25. [3] Lee, TH et al. (2012) International Immunopharmacology 13: 264 – 270. [4] Jung, M-R et al. (2012) Biochemical and Biophysical Research Communications 427: 236 – 241. [5] Park, CK et al. (2007) International Immunopharmacology 7: 1507 – 1516. [6] Lee, SI et al. (2010) Biomolecules & Therapeutics 18: 191 – 196.

PJ21 Inhibition of GIRK channels by extract of *Polygonum persicaria* and isolation of new flavonoids

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Polygonum persicaria L. is a morphologically extremely variable perennial plant, naturalized in various parts of the world. Previous phytochemical studies revealed the presence of stilbenes, flavonoids and phenolic acids in the plant. *In vitro* pharmacological studies demonstrated its antibacterial, antifungal, insecticidal and anti-inflammatory activities. The aim of the present work was the study of G protein-activated inwardly rectifying K^+ channel (GIRK) inhibitory activity of *P. persicaria*. The $CHCl_3$ extract of the plant exhibited high GIRK channel inhibitory activity at 0.1 mg/mL concentration. In the preparative experiment, the plant material was extracted with MeOH. The MeOH extract was subjected to solvent-solvent partition, affording *n*-hexane, $CHCl_3$ and aqueous extracts. The $CHCl_3$ extract was fractionated by VLC on RP-silica gel, and fractions obtained here were evaluated for GIRK modulatory activity. The most active fractions were subjected then to RP-HPLC, affording the isolation of the main compounds and a mixture containing the minor constituents. The pure compounds were identified by means of UV, HRMS and NMR spectroscopy as a new flavon (5,3',4',5'-tetramethoxy-6,7-methylenedioxyflavon) and three new flavonols (3-*O*-senecioyl-isorhamnetin; 3-*O*-angeloyl-isorhamnetin; 3,5,3',4',5'-pentamethoxy-6,7-methylenedioxyflavon). The isolated compounds were tested for GIRK inhibitory activity and found that neither separated, nor combined application of compounds modified the GIRK channel activity. However, a marked GIRK current inhibiting effect could be detected using the HPLC eluates containing the minor compounds. These results indicate the presence of electrophysiologically active agents among the minor compounds. **Acknowledgements:** This work was supported by grant OTKA PD 101432 and the European Union and co-funded by the European Social Fund TÁMOP-4.2.2/B-10/1 – 2010 – 0012 and TÁMOP -4.2.2.A-11/1/KONV-2012 – 0035.

PJ22 Total polyphenol content and assessment of antioxidant activity of selected medicinal plants

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The present work aims to assess the total polyphenols content and *In vitro* antioxidant activity of aqueous and methanol extracts of three selected medicinal plants used in traditional medicine; *Eucalyptus globules, Peganum harmala* and *Nigella sativa*. The antioxidant capacity was evaluated by applying two methods; ß-carotene bleaching assay and the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay. The MeOH extracts presented high levels of polyphenolic compounds (up to 333 ± 0.77 µg gallic acid equivalents (GAE)/mg plant extract. The antioxidant activity of the three selected medicinal plants was found to be dose dependent with polyphenols concentration. The alcoholic extracts displayed stronger antioxidant capacity than did aqueous extracts. Using the DPPH free-radical scavenging assay, the MeOH extract of *E. globulus* showed the highest antioxidant activity (IC_{50}= 14.8 ± 0.61 µg/ml). The test of β-carotene bleaching indicates that the MeOH extract of E. *globulus* showed the highest percentage of the antioxidant activity (69.9%). Results of this study demonstrate that leafs extracts of *E. globulus* possess strong antioxidant properties and therefore could be used in pharmaceutical industries.

PJ23 Anti-oxidant and anti-angiogenesis properties of *Terminalia bellerica* (Gaertn.) Roxb. and *Terminalia chebula* (Retz.) crude extracts

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Terminalia bellerica (Gaertn.) Roxb. and *Terminalia chebula* (Retz.), are native herbs of South East Asia. The fruits have polyphenol compounds which were reported to protect cells against oxidative damage at initiation and propagation stages of angiogenesis process in the formation of new blood capillaries of cancer cells. Therefore, the methanol and chloroform extracts of *T. bellerica* and *T. chebula* were investigated in order to assess their anti-oxidant and anti-angiogenesis activities. Chloroform and methanol extracts were further fractionated by using silica gel column chromatography with various solvent systems consisting of chloroform, chloroform-acetone, acetone-methanol and methanol to yield four fractions of chloroform extracts and four fractions of methanol extracts, respectively. The screening of anti-oxidant activities indicated that chloroform: acetone fractions from methanol extracts of both *T. bellerica* and *T. chebula* showed the highest anti-oxidant activities with an IC_{50} as 3.27 and 4.00 µg/mL in DPPH, 5.61 and 5.97 µg/mL in NO, and 194.57 and 172.34 mM Fe(II)/g in FRAP, respectively. We found that antioxidant capacity of *T. bellerica* and *T. chebula* related with total phenol content are 609.22 µg GAE/g and 538.75 µg GAE/g, respectively. For all fractions the inhibition of tumor angiogenesis on HUVECS cell was determinated. The results showed that methanol extracts show a higher inhibition on HUVECS cell than chloroform extracts and the highest inhibition showed acetone-methanol extract of *T. bellerica* with 84.3% and *T. chebula* with 68.6%. These results suggest that the methanol extracts of *T. bellerica* and *T. chebula* showed a higher anti-oxidant activity and provided the inhibition of tumor angiogenesis property on HUVECS cell than chloroform extract. And our results showed the highest antioxidant activity can be positively correlated with the highest total phenol content and also with the inhibitory effects on HUVECS cell.

PJ24 *In vitro* antioxidant activity, total phenolic content and phenolic composition of *Inula heterolepis*

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Inula heterolepis Boiss., a perennial herbaceous plant (15 – 40 cm) widespread throughout the Eastern Mediterranean region, is used topically in folk medicine against loss of appetite, headaches and hemorrhoids. To the best of our knowledge, a few studies were performed on *I. heterolepis* and in this study, total phenolic content, antioxidant capacity and phenolic composition of the plant were investigated for the first time (1 – 3). Total phenolic content of methanol extracts of aerial parts and roots of the plant was estimated by Folin Ciocalteu method. The aerial parts of the plant was seemed to contain higher amount of total phenolics compared to roots. Antioxidant activities of methanol extracts of flowers, leaves and roots of the plant were evaluated by DPPH radical scavenging and ABTS assays. All the investigated parts of the plant exhibited antioxidant activity in different concentrations. Flower and leaf extracts expressed higher antioxidant activities compared to root extract with lower IC_{50} values. With regard to the total phenolic content and antioxidant activity results, phenolic composition of the plant was investigated. Some phenolic compounds such as chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempferol were analyzed qualitatively and quantitatively in different parts of the plant by RP-HPLC. Various amounts of phenolics in different parts of the plant could be the main occasion for the determined antioxidant potential. **References:** [1] Davis, P.H. (1982) Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh. [2] Bohlmann, F. et al. (1982) *Phytochemistry*, 21(5):1166 – 1168. [3] Baytop, T. (1999) Therapy with Medicinal Plants in Turkey (Past and Present), 2nd Edition, Nobel Tıp Kitabevleri, Istanbul.

PJ25 Effects of *Epilobium* sp. main constituents and ellagitannins metabolites, urolitins, on proliferation and prostate specific antigen (PSA) secretion in prostate cancer cells (lncap)

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Taking into consideration the pathogenesis of hormone-dependent prostate cancer and application of *Epilobium* sp. extracts in traditional medicine in the treatment of prostate ailments, we have investigated and compared the activity of individual main Epilobium constituent (oenothein B, quercetin-3-*O*-glucuronide and myricetin-3-*O*-rhamnoside) on hormone-dependent prostate cancer cells (LNCaP) proliferation and prostate specific antigen (PSA) secretion. Due to high quantity of oenothein B in Epilobium sp. and the information from the literature about ellagitannins metabolites [1], we have examined the *in vitro* formation of urolithins from *Epilobium hirsutum* herb extract, rich in oenothein B, by human gut microbiota. The impact of urolithins on LNCaP cells has been also determined. Cell proliferation was measured using MTT and bisbenzimide (Hoechst 33258). PSA secretion was quantified by ELISA assays. Flutamid was used as positive control. Selected constituents were proven to be active in relation to LNCaP cells. However oenothein B was the strongest inhibitor of cells proliferation (IC_{50}= 7.8 ± 0.8 μM) and PSA secretion (IC_{50}= 21.9 ± 3.2 μM). Additionally, ellagitannins from *E. hirustum* extract was proven to be transformed by human gut microbiota into urolithins A-C. Urolithin C showed the strongest activity in the inhibition of cell proliferation (IC_{50}= 35.2 ± 3.7 μM), PSA secretion (reduced secretion from 325.6 ± 25.3 ng/ml to the level of 100.7 ± 31.0 ng/ml). Oenothein B content seems to be an important quality marker of *Epilobium* species but its active metabolites, urolithins, together with other extracts' constituents, may also determine the biological activity of these plant materials. Our results partly confirm the use of *Epilobium* sp. herbs in the treatment of prostate diseases. **Reference:** [1] Seeram et al. 2006. *J Nutr* **136**: 2481 – 2485.

PJ26 Near infrared spectroscopy (NIRS) for screening condensed tannins in a willow germplasm collection

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Willow belongs to the genus *Salix* and contains a number of species that are of great value as biomass crops.[1] Bark has been used throughout the world for centuries as an anti-pyretic and analgesic.[2] Willow is particularly rich in phenolic constituents, such as flavonoids, tannins and salicylates and these are of medicinal interest. This project aims to develop Near-Infrared Spectroscopy (NIRS) calibrations for tannin parameters of a large willow germplasm collection at the National Willow Collection at Rothamsted Research, Harpenden, UK. *In situ* thiolysis with benzyl mercaptan was used to characterise the tannins.[3] Samples were also screened by NIRS in order to develop calibration curves for predicting tannin content (CT) and structural parameters (mean degree polymerisation (mDP), procyanidins (PC), prodelphinidins (PD), cis and trans flavanols). Willow samples with high tannin contents will be tested for anthelmintic (nematicidal) activities. Willow samples collected in 2010 varied from 0.30 to 2.50 CT g/100 g dry matter; mDP from 4.1 to 26.1; PC/PD ratios from 14.8/85.2 to 97.7/2.3 and cis/trans ratios from 1.7/98.3 to 81.3/18.7. **Acknowledgement:** C. Klongsiriwet is grateful for a Royal Thai Government Scholarship. **References:** [1] Clapham, A.R. *et al.* (1987). Flora of the British Isles. 3rd Cambridge University Press. [2] Jassem, G.M. (2010) J. Saudi Chem. Soc. 14: 317 – 322. [3] Gea, A. *et al.* (2010) J.Agric. Food Chem. 59: 495 – 503.

PJ27 New flavonoid glucoside and other components taken from *Filipendula* genus plants

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The meadowsweet (*Filipendula ulmaria* Maxim.) is widely using plant in herbal medicine. Phenolics are one of the most important groups of nature compounds defining a pharmacological action of *F. ulmaria*. At the same time there are other widespread plants of *Filipendula* genus which can be used as a source of herbal medicines. The research of a chemical structure of these plants is necessary for identification of close species within the *Filipendula* genus. The separation of extract taken from *F. ulmaria* shoots was conducted by chromatographic methods. As a result forty nine substances were isolated including 48 known and a new compound named ulmarioside. Investigation of ulmarioside by complex of spectral methods (UV, IR, MS, NMR) allow to describe its structure as a quercetin-4′-*O*-α-rhamnopyranosyl-(1 – 6)-β-glucopyranoside (quercetin-4′-*O*-rutinoside).

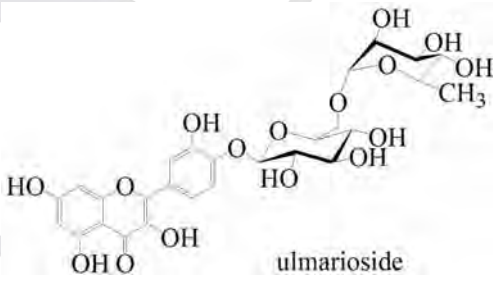

Fig. 1

Some components such as 1,3-di-*O*-, 3,4-di-*O*-, 3,4,5-tri-*O*-caffeoyl-, 3-*O*-, 5-*O*-galloyl quinic acids, 1-*O*-caffeoylglucose, quercetin-3-*O*-gentiobioside, catechins (C, EC, EGC, EGCG) and procyanidins (B_1, B_2) were identified in *F. ulmaria* for the first time. The chemical composition

research was done also for other plants of this genus such as *F. denudata*, *F. palmata*, *F. vulgaris* and the first time for *F. camtschatica* and *F. stepposa*.

Tab. 1: The variation in contents of bioactive substances (mg/g) in Filipendula genus plants (F. ulmaria, F. denudata, F. palmata, F. vulgaris, F. camtschatica. F. stepposa)

Part of plant	Flavonoids	Ellagitannins	Procyanidins	Catechins
Flowers	19.11 – 74.05	40.01 – 207.31	-	-
Leaves	18.35 – 43.15	9.15 – 52.13	20.31 – 37.05	18.63 – 103.14

Obtained data let suggest that ellagitannins tellimagrandin I_2, rugosins E_1, E_2, and flavonoids spiraeoside and kaempferol-4′-*O*-glucoside can be considered as chemotaxonomic markers in *Filipendula* genus, apart from the previously proposed rugosin D. The authors acknowledge for the financial support provided by the Russian Foundation of Basic Research under Project 12 – 03 – 31547.

PJ28 Essential oil of *Myrica rubra* Sieb. et Zucc. inhibits proliferation of HCT-8 tumor cell lines

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Natural sources are being explored for their potential to combat severe side effects caused by increasing resistance of mammalian tumor cells to chemotherapy. The widespread use of *Myrica rubra* Sieb. et Zucc. in traditional medicine motivated us to study its potential anticancer activity. In Chinese, Japanese and Taiwan traditional medicine, *M. rubra* is used as an astringent, antidiarrheic, analgesics and an antidote. We aimed to investigate the activity of *M. rubra* essential oil extracted by hydrodistilation and of leaves extracted separately in distilled water, ethanol, ethyl acetate, and terc-butyl-methylether. Together with the oil and leave extracts, we tested particular phenolic compounds previously reported for their biological activity, such as myricetin, myricitrin, and epigallocatechin 3-*O*-gallate presented in *M. rubra* leaves. Antiproliferative activity was monitored by xCELLigence Real Time Cell Analyzer against Human ileocecal adenocarcinoma cell lines HCT-8. GC x GC/MS analyses were performed to detect terpenoid compounds in the essential oil.

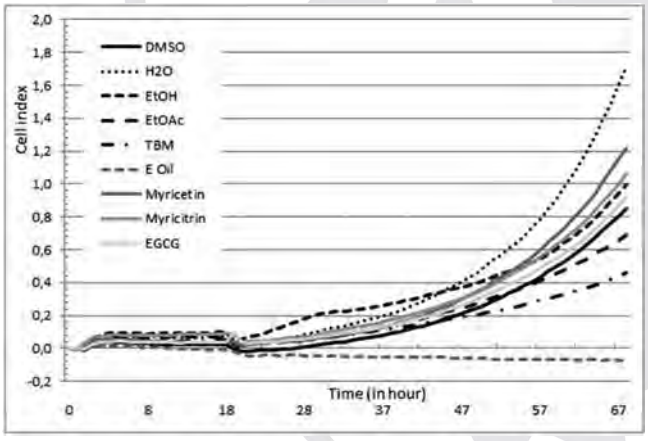

Fig. 1: Antiproliferative activity of *M. rubra* leaves against HCT-8 tumor cell lines monitored by xCELLigence Real Time Cell Analyzer. Extracts and essential oil were tested at concentration 50 µg/ml, phenolic compounds at concentration 5 µM. A solvent Dimethylsulfoxide (DMSO) was applied as a negative control. (H2O – aqueous extract, EtOH – ethanol extract. EtOAc – ethyl acetate extract, TBM – terc-butyl-methylether extract. E oil – essential oil, EGCG – (-)-Epigallocatechin gallate)

Antiproliferative tests reveal significantly higher activity of essential oil compared to leave extracts and particular phenolic compounds (see Figure). Subsequently we tested the oil in different concentrations and found 91.46% inhibition at concentration of 10 $\mu g^{*}ml^{-1}$. Furthermore, the essential oil shows no toxicity to hepatic cells. GC x GC/MS analysis proves the presence of β-Caryophyllene, and α-Humulene as major compounds in *M. rubra* oil. Both were previously reported as potential anticancer agents. In conclusion, our results indicate that terpenoids possess stronger inhibition to tumor cells compared to phenolic compounds. Our further investigation of *M. rubra* essential oil can reveal new compounds with potential anticancer activity. This work was supported by KONTAKT LH12165 project granted by Ministry of Education, Youth, and Sports of the Czech Republic.

PJ29 Separation of phenylethanoid glycosides from two *Lantana* species by High-Speed Countercurrent Chromatography

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The genus *Lantana* (Verbenaceae) comprises approximately 150 species, distributed in tropical and subtropical regions. *Lantana fucata* Lindl. is a small shrub used in the Brazilian traditional medicine as a carminative, anti-inflammatory, and to treat colds and bronchitis. In a previous work from our group, the alcoholic extract from this plant and Fucatoside C, isolated from it, showed significant *in vitro* antiinflammatory activity (Julião et al., 2009). *Lantana trifolia* L. is used in Brazilian folk medicine for respiratory disorders and as sedative. Its antiinflammatory and analgesic activities were also demonstrated by us (Julião et al., 2010). In the present study, *n*-butanol extracts from leaves of *L. fucata* and *L. trifolia* were fractionated by HSCCC as a new strategy to isolate the active compounds for biological investigations. CCC is a technique based on liquid-liquid extractions and uses no solid stationary phase. The choice of solvent system was performed by test-tube partition test. The solvent system chosen was ethyl acetate/*n*-butanol/water (1.0:0.2:1.0, v/v) for both *n*-butanol extracts. The isocratic elutions (normal phase) were performed on HSCCC equipped with a multi-layer coil, conditions as follows: column 80 mL, 1.6 mm id, flow rate 2.0 mL/min (4.0 mL/tube), 60 tubes, 850 rpm, temperature 26 °C, loop injection 5.0 mL, wash off = 80 mL. Stationary phase retention (*Sf*) was 75%. Two phenylethanoids – Numioside A (23 mg, 77.4%) and Fucatoside C (31.9 mg, 77%) were isolated from *L. fucata*, and two from *L. trifolia*: Verbascoside (29.5 mg, 80.5%) and Apiosylverbascoside (39.3 mg, 81.8%). Purities were determined by HPLC. All substances were isolated in one-step separation from *circa* 400 mg of each extract, in a much more efficient and costless process than the other techniques used in the previous works. Support: CAPES and CNPq. **References:** [1] Julião LS; et al., *J. Nat. Prod.*, 72, 1424 – 1428, 2009; [2] Julião LS; et al. *Phytochemistry*, 71, 294 – 300, 2010.

PJ30 Ellagitannin-enriched fraction from *Fragaria vesca* leaves induces G2/M cell cycle arrest in the human hepatocellular carcinoma cell line HepG2

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The hepatocellular carcinoma has a poor prognostic and a lower survivor rate. The inefficacy of the conventional therapies highlights the importance of discovering new compounds with anti-tumor effects. Previous studies performed in our laboratory demonstrated that a hydroalcoholic

extract of *Fragaria vesca* L. leaves affects cellular proteolitic pathways through the reduction of proteasome activity and modulation of autophagic machinery, mechanisms that are therapeutic targets in cancer treatment. Thus, the aim of this study was to evaluate the anti-cancer properties of an ellagitannin-enriched fraction (EEF) from *Fragaria vesca* leaves (characterized by HPLC/PDA/ESI-MS^n), in human hepatocellular carcinoma cells (HepG2). To evaluate half-maximal inhibitory concentration (IC-50) for cell viability, the cells were treated for 24 h and then assayed by resazurin. IC-50 (113 μg/mL) and lower concentrations were used to evaluate the effect of the fraction on cell cycle. As evidenced by propidium iodide addition, after 24 h of treatment, EEF induced cell cycle arrest at G2/M checkpoint, and this effect was not reverted after 48 h. Cell cycle analysis also suggests that apoptotic cell death is not responsible for the cytotoxic effects. Moreover, by western blot we observed an increased in the ratio of expression of LC3II/LC3-I. LC3 is a marker of autophagossomes, and the conversion of LC3-I to LC3-II is an evidence of an accumulation of these structures, thus suggesting a strong modulation of the autophagic process, by increasing autophagy or through inhibition of the autophagic flux. In conclusion, EEF promotes cell cycle arrest at G2/M checkpoint and modulates autophagy, processes that are targets for therapeutic strategies in oncological diseases. **Acknowledgements:** FCT and POFC/FEDER for financial support. Research supported by FCT PhD fellowship SFRH/BD/72918/2010, projects PTDC/SAU-FCF/105429/2008 and PEst-OE/SAU/UI0177/2011.

PJ31 Australian *Eucalytpus* kinos – Past, Present, Future

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Kinos are tannin-rich, mostly red-coloured wood exudates. Their characteristic astringency has made them a popular remedy in a number of traditional systems of medicine around the world. The most abundant kinos of Australian origin are that of *Eucalyptus* species which have played an important role in the traditional medicines of Australian Aboriginal people and were also a valued source of antibacterial and astringent agents for early European settlers.

Fig. 1

For this study a detailed literature review on a large number of Western Australian *Eucalyptus* kinos was carried out to collate information on their traditional medicinal use as well as any published chemical data. Whilst it was possible to find mainly historical and ethnopharmacological documentation it appears that not much is known about their chemical composition and potential pharmacological effects. To establish some baseline data these kinos were therefore analysed for their total phenolics and total tannin content as well as their relative amounts of hydrolysable and condensed tannins. They were also classified in accordance with Maiden's traditional kino categories and assessed for their antimicrobial properties against some Gram positive and Gram negative bacteria as well as a yeast species using well plate diffusion assays. Based on the obtained data possible correlations between chemical composition and antimicrobial activity are drawn and individual kinos are identified which warrant more research in the future.

PJ32 Inter- and intra-accession variation of isoflavones in red clover plants

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The literature contains a surprising diversity of methods for extracting and analysing isoflavones in red clover. The objective of this study was to measure the extent of variation between red clover varieties and within varieties in terms of isoflavone composition and concentrations. However, few reports exist on how to store samples after harvesting and how to achieve quantitative extraction efficiency. Extraction methods were compared using freeze-dried plant samples with those that were homogenised in methanol. This information is needed for first preserving and then comparing large numbers red clover leaflets from pot and field experiments. Isoflavones were determined as aglycones after acid hydrolysis, as aglycones are bioavailable. Two accessions of wild red clover plants were used for this experiment. HPLC profiles (without acid hydrolysis) were run to determine any changes during the preservation processes. Homogenisation in methanol generated higher isoflavone contents and lower standard errors than freeze-drying of plant samples. Some of the major isoflavones were quantified; genistein ranged from 57 to 151 μg per gram of fresh leaflets; formononetin from 115 to 1221 μg/g and biochanin A from 0 to 5625.0 μg/g. Interestingly, in the accession with undetectable levels of biochanin A the major isoflavone was tentatively identified as prunetin. Thus there were significant differences in isoflavone concentrations and composition between and within varieties.

PJ33 Cytoprotection of *Ulva rigida* against hydrogen peroxide-induced apoptosis

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Ulva rigida (Chlorophyta) has been suggested to contain cytoprotective agents against oxidative stress because of its antioxidant activity. However the antioxidant mechanisms of *U. rigida* have not yet been explored. In the present study, the protective effect of *U. rigida* against oxidative stress was evaluated, and the underlying molecular mechanisms were investigated in HeLa cervical carcinoma cells. Quantification of apoptosis by flow cytometry indicated that *U. rigida* extract inhibited H_2O_2-induced apoptosis in HeLa cells. In addition, using $DiOC_6(3)$ localization as a measurment of mitochondrial integrity, we demonstrated that *U. rigida* extract prevented the decline of membrane mitochondrial potential ($\Delta\Psi$) induced by H_2O_2 stress. Western blot analysis revealed that the level of the pro-apoptotic protein p21 Bax decreased in H_2O_2 treated cells compared to the untreated cells, while it was consistely detected in cells co-treated with H_2O_2 and *U. rigida* extract. These can be associated to a cleavage, following H_2O_2 stress, of the full-length form of p21 Bax into a p18 Bax form accepted to be more potent in disrupting mitochondrial integrity. This study provided a possible explanation for the antioxidant activity of *U. rigida,* and implied an application of this alga for the therapy of diseases involving oxidative stress.

PJ34 Methanolic Leaf Extract of *Parkia biglobosa* Protects against Doxorubicin-induced Cardiotoxicity in rats

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Parkia biglobosa aqueous-methanolic leaf extract, PBE (DER-10:1) was investigated for its effects on doxorubicin-induced cardiotoxicity, phytochemical constituents and *in vitro* antioxidant activity. Adult albino (200 – 220 g) rats were divided into seven groups (n = 6). The control and the doxorubicin-challenged, untreated groups received distilled water orally for 14 consecutive days while on the 13th day, the latter received single dose of doxorubicin (15 mg/kg i.p). The pretreated groups received

ramipril (10 mg/kg b.w) or *PBE* (25 mg/kg, 50 mg/kg, 75 mg/kg and 200 mg/kg b.w) by gavage throughout and later were given doxorubicin (15 mg/kg i.p) on the 13th day. Animals were sacrificed 20 hours after the last administration, the heart homogenate and serum were analysed for glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), glutathione-s-transferase (GST), glutathione peroxidase (GPX), catalase (CAT), creatine kinase MB (CK-MB), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides (Trig). Results revealed the presence of saponins, tannins, cardiac glycosides, total flavonoid (256.86 mg Quercetin equivalent) and total phenol (144.18 mg gallic acid equivalent), significant reduction in the iron sulphate induced formation of MDA. Doxorubicin caused significant elevations ($P < 0.05$) of cardiac MDA, serum CK-MB, LDH, AST, Trig and LDL while causing significant reduction in the levels/or activities of cardiac GSH, GPX, GST, SOD and serum HDL. Pretreatment with PBE reversed these parameters to near normal levels. The protection offered compared well with that of ramipril. The results of this study reveal that *PBE* can protect against doxorubicin-induced toxicity and that the protection might be via antioxidative and antihyperlipidemic mechanisms Key words: *Parkia biglobosa (Fabaceae);* cardiotoxicity; doxorubicin; antioxidative; antihyperlipidemic

PJ35 Antioxidant and antidiabetic activity of fermented *Youngia sonchifolia* Maxim leaf tea

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This study was carried out to investigate antioxidant and antidiabetic activity as well as decrease a bitter taste of fermented tea from *Youngia sonchifolia* Maximleaf tea by using the lactic ferment. The pH contents and the titratable acidity contents of fermented tea from *Youngia sonchifolia* Maximleaf tea each showed the *Lactobacillus brevis* (LB) and *Leuconostoc mesenteroides* (LM) treatments were critically low. The color intensity of fermented tea increased. When sensory test were conducted, fermented *Youngia sonchifolia* Maximleaf tea has a synthetic and pleasant taste. While a bitter taste was lower, flavor and overall acceptability of LB treatment were significantly higher than other treatment. Free amino acid related in a bitter taste of the LB treatment decreased and contents of organic acids were markedly increased through fermentation process of *Youngia sonchifolia* Maxim. The DPPH radical scavenging activity of fermented tea showed higher LB treatment than control. And The α-glucosidase inhibition of fermented tea showed higher LP treatment than control. On the basis of these results, we conclude that fermented tea from *Youngia sonchifolia* Maximleaf tea could help decrease a bitter taste through the process of fermentation and be applied as medicinal and edible resources in an industrial area due to their effective antioxidant and Antidiabetic activities.

PJ36 Bioenergetic and antioxidant effects of a fresh leaf extract from *Cynara scolymus* L.

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Fresh leaf artichoke extracts contain high amounts of cynarin and related caffeoylquinic acids which possess high antioxidant activity. We have investigated mitochondrial stimulation and confirmed radical scavenging activity of cynarin, the lead compound of the special extract [15 – 30:1] from fresh leafs instead of dried material from *Cynara cardunculus* var. *scolymus* (L.) Benth., and its main bioactive metabolites caffeic acid, ferulic acid, dihydroferulic acid and their amide derivatives formed after administration of the fresh leaf artichoke extract to humans. Neuroblastoma cells were incubated with 100 and 200 μM cynarin or with their main metabolites at similar concentrations. All compounds increased mitochondrial respiration as assessed by NBT (Nitro Blue Tetrazolium) reduction by 3 to 6 fold. Activity of complex I (iron sulfur cluster N2) and complex IV (cytochrome-c-oxidase unit 5A) were equally stimulated after administration of cynarin and its derivatives to the same extend. Even under baseline conditions, NO formation was increased twofold, whereas the generation of superoxide anion radicals and peroxynitrite was reduced by almost 50% compared to vehicle treated control cells. Cynarin exhibited potent hydroxyl radical scavenging activity (EC_{50}= 50 μM), whereas caffeic, ferulic and dihydroferulic acid were even more potent (EC_{50}~25 μM). More importantly, the amide derivatives like melatonin and indolepropionamide, that have a unique bioavailability (Poeggeler et al., 2010; Poeggeler, 2013) due to their high amphiphilicity and therefore possess high bioactivity in vivo, were also quite potent (EC_{50}~75 μM). **References:** [1] Poeggeler B., Sambarmurti K., Siedlak S.L., Perry G., Smith M.A., Pappolla M.A.: A novel endogenous indole protects rodent mitochondria and extends rotifer lifespan. PLoS ONE 5 (4): e10206, 2010. [2] Poeggeler B.: Die Heilkraft der Artischocke bei Verdauungsproblemen und Leberstoffwechselstörungen. Zeitschrift für Phytotherapie 34 (Suppl. 1): S25, P12, 2013.

PJ37 Polyphenolic fingerprints of different organs of *Rosa hybrida* cv. 'Jardin de Granville'

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Rosa hybrida cultivar 'Jardin de Grandville', a delicate clear pink flower, was selected by Parfums Christian Dior to enter in some luxury cosmetic formulations. This cultivar has been selected not only for its beauty but also for its resistance to diseases. In order to characterize the phytochemical content of this plant, responsible of its singularity, different plant organs are investigated. Analyses are focused on the polyphenol family, widely described in the literature for their large diversity of functions in the plant (coloration, antioxidant activities, defense molecules against diseases and pathogens...). The investigation of these polyphenolic compounds in *Rosa hybrida* cv. 'Jardin de Grandville' is of a great interest, considering its high level of selection. The phytochemical content of 16 plant organs is evaluated: winter woods, shoots, early buds, buds before flowering, flowers (first flowering period, spring), four flower parts analyzed separately (petals, sepals, receptacles and stamens), leaves, summer woods, summer shoots, buds before flowering, flowers (second flowering period, summer), roots and fruits. Reversed phase UHPLC technique coupled with DAD and ESI-UHR-Q-TOF mass spectrometry was used to manage the analyses. Combining all the information collected (absorbance maxima, accurate mass and molecular formulae) and thanks to comparison with reference standards, several phenolic compounds have been characterized like tannins derived from quinic, gallic and ellagic acid, quercetin derivatives and kaempferol derivatives. Statistical analyses (PCA, HAC and ANOVA) are applied to this data set to point out similarities or differences between the different parts of the plant. A clear separation between vegetative and reproductive organs is obtained. Thus, compounds like kaempferol derivatives are representative of the reproductive organs whereas catechin derivatives are more abundant in the vegetative parts.

PJ38 The content of procyanidin trimers of types A and B in some *Vaccinium* species and cultivars

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The flavonoids procyanidin trimers of A and B types known as antidiabetic and antiobesic compounds [1] were detected in some *Vaccinium* species, their content has been determined only in blueberry/bilberry plant leaves and fruits but not in the stems. The aim of this study was to compare their content in different parts of *V. myrtillus* and *V. corymbosum* cultivars. We collected stems (60 samples) and leaves (30) of *V. myrtillus* and *V. corymbosum* cultivars 'Ama' and 'North Blue' from different locations in Estonia around the year, and estimated the concentration of bioactive polyphenols by reversed phase HPLC-MS; procyanidins were identified by their spectra and quantified using catechin as a standard. The content of oligomeric procyanidins was significantly higher in the stems than in the leaves, and a clear seasonal variability was also detected. The seasonal/geographic variation in the content of procyanidins was nearly tenfold. The concentration of type A procyanidin was drastically higher in the *V. myrtillus* plants than in hybrid blueberries, while the differences in procyanidin B concentrations were less prominent. The level of procyanidins A and B was rather equal in stems and leaves of *V. myrtillus* collected from different locations, whereas the content of A-type isomer was higher. The highest content of procyanidin A in *V. myrtillus* stems was more than 3000 times higher than the lowest

content in the leaves of *V. corymbosum* cultivar 'Ama'. No clear seasonal dynamics in procyanidins content in either *Vaccinium* spp. stems or leaves was found, but the ratio of the highest and lowest values for stems, collected in the same location at different times was between 3 and 18, whereas the highest contents of procyanidin A in the stems of *V. myrtillus* were over 1% of the dry matter. Conclusion: the best sources of procyanidin trimers of type A and B are the stems of *V. myrtillus*. **Reference:** [1] Nakahara K. et al. (2001) U. S. States Patent 6,294,190 B1, September 25.

PJ39 Plant species to control diabetes used in counties in the extreme upper Basin Platinum, Alto Pantanal Portion, Mato Grosso, Brazil

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Diabetes is a disease which progresses within and between age groups. Today the local people use medicinal plants as an alternative to health care. Define plants to every illness, as well as diabetes. This work shows plants used for diabetes at the upper end of the Platina Basin (P), near the southern part of the Amazon Basin (A). The study, part of Project PLAMED, included 14 municipalities of Alto Pantanal Basin Platinum (P), Mato Grosso, Brazil. In the north of P has a range of transition basins (PA = T) and going over has been the beginning of the Amazon Basin (A). Data were collected by interview in 2005, with informants recognized competence in the subject and indicated by the local community. In two counties there was no appointment of species to treat diabetes. In others (12), were given 1 – 4 esp/mun. totaling 35 indications showed 18 species used in P for diabetes: "Abacaxi"(*Ananas comosus* (L.) Merr.), "Calunga"(*Simaba ferruginea* A. St.-Hil), "Caferana"*(Vernonia polyanthes* Less), "Cipó da Amazônia"(*Banisteriopsis caapi* (Spruce) Morton), "Carambola"(*Averrhoa carambola* L.), "Douradinha"(*Palicourea xanthophylla* M. Arg,) "Insulina"(*Cissus verticillata* (L.) Nicholson & C.E. Jarvis), "Quebra-pedra"(*Phyllanthus niruri* L.), "Sai-de-mim"(?), "Tiririca"(*Cyperus rotundus* L.), "Caju-do-campo"(*Anacardium humile* A. St.-Hil), "Cebola-roxa"(*Allium cepa* L.), "Pedra ume-ca-á"(*Myrcia sphaerocarpa* DC.), "Açoita-cavalo"(*Luehea divaricata* Mart. et. Zucc); G2) 3 common between P and A: "Urucum"[*Bixa orellana* L.], "Pata-de-vaca"[*Bauhinia* sp], "Jucá"[*Caesalpinia ferrea* Mart.; G3) one kind was triple-joint (P, AP, A): "Carqueja"[Baccharis sp]. The most frequently mentioned were "Pata-de-vaca" (6/12) and "Carqueja" (7/12), and the others had one appointment each. The target plants are concentrated in two species and disperse in the others. Among the 18 species, the "Cebola" has more and "Douradinha" "has less research related to diabetes, which were available online at abr2013

PJ40 Phenolic compounds and antioxidant activity of *Taraxacum gracilens* Dahlst. aerial parts

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The genus *Taraxacum* is a member of the family Asteraceae, subfamily Cichorioideae, tribe Lactuceae. The name is derived from the Greek words 'taraxis', for inflammation, and 'akeomai' for curative (1 – 2). The total number of *Taraxacum* in Turkey at present is 43 species (3). In folk medicine, this genus has been utilized for the treatment of various diseases such as dyspepsia, heartburn, spleen and liver complaints, hepatitis and anorexia (1). Previous phytochemical investigations have shown that *Taraxacum* species contain sesquiterpene lactones, triterpenes, phytosterols, flavonoids, lignans, coumarins, phenolic acids, beta-carboline alkaloids, indole alkaloids, carotenoids (1 – 2). *Taraxacum gracilens* Dahlst. is a perennial herb growing in the European part of Turkey (3). In this work, we report the isolation of flavonoids, coumarins, phenolic acids from the aerial parts of *T. gracilens* by repeated column chromatography and prep. TLC, and structure determination by spectral analyses. The polyphenolic content and antioxidant activities of the petroleum ether, CH_2Cl_2, EtOAc and BuOH fractions of the MeOH extract from the aerial parts of *T. gracilens* were investigated. EtOAc and CH_2Cl_2 fractions showed the highest antioxidant activity, respectively. This is the first report on the isolation of phytochemical constituents and the antioxidant activity of the aerial parts of *T. gracilens*. **References:** [1] Schütz, K., Carle, R., Schieber, A. (2006). Taraxacum- a review on its phytochemical and pharmacological profile. Journal of Ethnopharmacology, 107, 313 – 323. [2] Leu, Y., Shi, L. ve Damu, A.G. (2003). Chemical constituents of *Taraxacum formosanum*. Chemical Pharmaceutical Bulletin, 51(5), 599 – 601. [3] Van Soest, J. L. (1975). *Taraxacum* Wiggers. İçinde P.H. Davis (Ed.), *Flora of Turkey and the East Aegean Islands Vol 5*. Edinburgh: University Press; 778 – 812.

PJ41 Proanthocyanidin-enriched extract of *Rumex acetosa* L. inhibits the *in vitro* adhesion of *Porphyromonas gingivalis* to KB cells and decreases the biofilm formation

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Porphyromonas gingivalis is a gram-negative periodontal pathogen, which plays a significant role in the progression of chronic periodontitis and may even be involved in the development of other cardiovascular diseases. The first critical step for the initiation of periodontitis is the specific adhesion of bacteria to tissue cells. By forming complex biofilms, anaerobic bacteria as *P. gingivalis* evade the hosts immune defense. In contrast to conventional antibacterial therapy approaches, an acetone-water extract of *Rumex acetosa* L. (RA1) [1] was tested for *in vitro* antiadhesive activity on KB cells by flow cytometrie in order to initiate a development against the first step of the infection. Bacteria, preincubated with RA1 (100 µg/mL) showed 25% less adhesion to KB cells. By coincubating bacteria, KB cells and RA1 (10 µg/mL) a significant decrease of adhesion of 37% was observed. Structure-activity relationship of relevant polyphenols from RA1 indicated that proanthocyanidins are responsible for these effects. Especially epicatechin-3-O-gallate-(4β->8)-epicatechin-3-O-gallate (20 µM: 61%; 30 µM: 96% inhibition) significantly reduces the adhesion of *P. gingivalis* to KB cells. Epicatechin-3-O-gallat has a lower antiadhesive activity (20 µM: 21%; 30 µM: 25% inhibition). Non-galloylated flavan-3-ols had no effect. Besides the inhibition of bacterial adhesion, the influence of RA1 on the biofilm formation was investigated. We could detect a significant inhibition of biofilm formation, after staining the biofilm with crystal violet (RA1 100 µg/mL, 51% inhibition). **Reference:** [1] Bicker J, Petereit F, Hensel A (2009) Proanthocyanidins and a phloroglucinol derivative from Rumex acetosa L. Fitoterapia. 80(8); 483 – 95.

PJ42 Variation of chemical composition of *Epilobium angustifolium* during fermentation

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Infusion from fermented *Epilobium angustifolium* L. herb (Ivan tee) is traditionally consumed in Russia for the treatment of stomach ulceration, gastritis, and sleeping disorders. It has been used in folk medicine to treat a variety of ailments such as benign prostate hyperplasia and the associated problems of micturition. Flavonoids and tannins, particularly oenothein B, dimeric hydrolyzable ellagitannin seemed to be responsible for these activities. The aim of the study was evaluation of chemical composition of *E. angustifolium* during fermentation procedure. Aerial parts of *E. angustifolium* were harvested from plantation in Mikkeli (Finland) in different vegetation phases and fermented as described [1]. Maximal concentration of tannins and oenothein B was observed at the beginning of flowering, while flavonoids and hyperoside at the flowering phase. After fermentation the concentration of total tannins, hyperoside and oenothein B were decreased in 35%, 42% and 75% respectively, *although* content of total flavonoids was not changed significantly. The minimal decrease was observed at 35 – 40 °C fermentations, especially for Oentohein B, as a one of the key compound. **References:** [1] Shikov A.N. et al. J Agric Food Chem 2006; 54: 3617 – 3624 **Acknowledgement:** the study was supported by project SPECICROP, ENPI CBC 2007 – 2013

PJ43 Bioassay-directed isolation of phytoestrogens from *Eucommia ulmoides*

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Recently, phytoestrogens have drawn growing attention, as increasing evidence reaveled a possible role in preventing a number of diseases, such as the hormonally dependent cancers. Some secondary metabolites from plants may act as excellent natural phytoestrogens [1]. *Eucommia ulmoides*, the sole species of the *Eucommia* genus (Eucommiaceae), is a large medicinal hardwood native to China and now distributed widely in South Korea and China. The tree has extensively been used in Korean and Chinese medicines as a tonic to treat diabetics, lower blood pressure, prevent fatigue and miscarriage, and strengthen the liver and kidneys [2]. In this work, the MeOH extracts of the *E. ulmoides* root were successively fractionated into *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH and the remaining H_2O fractions. The *n*-BuOH soluble fraction, which showed significant phytoestrogenic activity, by phytoestrogenic activity-directed column chromatography was subdivided to yield five phenolics, including kaempferol, quercetin, apigenin, naringenin and ellagic acid. The structures of the isolated compounds were elucidated on basis of spectroscopic methods. The phytoestrogenic properties of the constituents were determined by an assay with the human cortical cell line HCN 1-A [3]. This study demonstrates that the five phenolics from *E. ulmoides* root might be valuable candidates for the development of phytoestrogenic drugs. **References:** [1] Cornwell T. et al. (2004) Phytochemistry 65: 995 – 1016. [2] Si CL, et al. (2011) Planta Med 77: 1338 – 1338. [3] Occhiuto F. et al. (2008) Phytomedicine 15: 676 – 682. **Acknowledgements:** This work was financed by National Natural Science Foundation of China (31170541, 31000279), Natural Science Foundation of Tianjin City (13JCZDJC), Program for New Century Excellent Talents in University (NCET-10 – 0951), Foundation (2012IM002) of Key Lab of Industrial Fermentation Microbiology of Ministry of Education & Tianjin Key Lab of Industrial Microbiology, China.

PJ44 HPLC method for quantification of *Camellia sinensis* catechins entrapped in liposomal carriers

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Many natural products of commercial importance in food and pharmaceutical sectors, are complex mixtures of substances. Examples of herbal substances with claimed health benefits include the phenolic compounds. *Camellia sinensis* (green tea) is a plant with a high concentration of polyphenols with several biological properties such as antioxidant, antitumor, antimicrobial, etc. However, the biological effect can be drastically diminished or lost after oral administration, due to low solubility of the compound in the gastrointestinal tract (low absorption), and the effect of presystemic metabolism. The entrapment of bioactive compounds in liposomes has been proposed as a promising method to overcome bioavailability restrictions. However, depending on the properties of the phenolic substance, a strong physicochemical interaction with the lipid bilayer can emerge, which makes the quantification of the entrapment efficiency difficult. The aim of this work was to develop and validate a HPLC method for quantification of green tea (GT) catechins epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) encapsulated in liposomal lipid carriers. Liquid, spray dried and freeze dried compositions containing lecithin:cholesterol: GT lyophilized extract:surfactant (8:1:2:2) were prepared and used for validation of the HPLC method. Samples were extracted with ultrapure water and with a mixture of water plus triton X-100 (in order to open the lipid vesicles), and analysed using a reversed phase C-18 column. The results were compared with the non encapsulated lyophilized extract of GT and standard substances. A significant change in the HPLC profiles was observed for the encapsulated samples, which was linked to the strong molecular interaction between the GT polyphenols with the phosphatidylcholine lipid bilayers. High encapsulation efficiencies were observed for liquid and dried formulations, which were dependent of the polyphenol moiety (EGCG > ECG).

PJ45 Activity-guided discovery of antioxidative compounds in bark extract of *Garcinia buchananii*

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Reactive oxygen species (ROS) are well-known to be generated as by-products of the normal cell aerobic respiration that is essential to life and play a crucial role in cell signaling and homeostasis. However, overproduction of ROS causes undesirable oxidative stress which is associated with degenerative and chronic diseases such as, e. g. cardiovascular disease, cancer, diabetes, neurodegeneration and is involved in the process of aging. About 400 species are known of the genus *Garcinia*, familiy Guttiferae, in which extracts and pure compounds from *Garcinia* species exhibited forms of biological activity such as anticancer, antimicrobial, anti-inflammatory and antioxidant properties. In order to identify antioxidant components in the stem bark extract of *G. buchananii* tree, were performed activity-guided fractionation using in vitro tests like H_2O_2 scavenging, oxygen radical absorbance capacity (ORAC) as well as trolox equivalent antioxidant capacity (TEAC) assays. The most active principles were identified by means of UPLC-TOF-MS^e analysis, 1D- and 2D-NMR and CD spectroscopy as a series of 3,8''-linked biflavanones and flavanone-*C*-glycosides (Figure). The H_2O_2 scavenging, TEAC and ORAC assays demonstrated that these natural products have an extraordinarily high antioxidative power compared to common antioxidants like (epi)-catechin, rutin, quercetin, naringenin or ascorbic acid. Finally, reconstitution experiments were done to evaluate additive and/or synergistic effects of the antioxidative compounds identified. These findings demonstrate that *G. buchananii* bark extract is a rich natural source of biflavanone and flavanone glycoside antioxidants.

PJ46 Biotransformation of blueberry anthocyanins to bioavailable phenolic compounds by *Lactobacillus*

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Blueberries are one of the most anthocyanin-rich fruits, containing more than 25 forms of anthocyanins. This high anthocyanin content may contribute to the health benefits of blueberries against cardiovascular diseases, diabetes and cancers. However, the bioavailability of anthocyanins is relatively low, meaning that intact anthocyanins are unlikely to act as local antioxidants. Anthocyanin metabolites, such as phenolic acids, are easier to absorb, and are thus more bioavailable. This study has investigated the biotransformation of blueberry anthocyanins and a standard anthocyanin, malvidin-3-glucosides, into metabolites in the presence of *Lactobacillus plantarum* and *Lactobacillus sakei* at pH 3.4, 4.2, 5.9 & 7.0 and incubated anaerobically at 37°C for up to 24 h. Samples were centrifuged and the supernatants were analysed using semi-preparative liquid chromatography. The metabolites were identified using high performance liquid chromatography-mass spectrometry (LC-MS). Malvidin glycosides were found to completely hydrolyse at pH 4.2 and 5.9 after 24 h of incubation. The anthocyanins were quite stable under acidic conditions (pH 3.4) but were highly unstable at neutral pH and disappeared after 8 h at pH 7.0 by both chemical and microbial degradation. Similar results were obtained when testing the same strains with blueberry samples, although most of the anthocyanins were not fully transformed due to their high initial concentrations. The primary metabolites were phenolic acids and aldehydes such as syringic acid, gallic acid and protocatechuic acid (Figure 1). These metabolites showed a greater antioxidant capacity than the anthocyanins themselves as the antiradical power was found to increase in the order, malvidin-3-glucoside < syringic acid < protocatechuic acid < gallic acid. Thus, the metabolites of blueberry anthocyanins may be mainly responsible for the antioxidant activity of blueberries against chronic diseases, which then contribute to a healthy heart.

PJ47 Chemical composition and biological activities of extracts from an endemic *Salvia cilicica* Boiss.

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The genus *Salvia* L. (Lamiaceae) comprises about 900 species around the world, while it is presented with 89 species and 93 taxons in Turkey, approximately half of which is endemic (1,2). Anatolia is the major gene center in Asia. The previous studies on this genus were about the isolation of their terpenic and flavonoid compounds and some biological activities. An endemic species in Adana-Pozanti, *Salvia cilicica*, has only a limited number of studies in the literature regarding its chemical composition and biological activity. In our previous study, we presented the isolation of the terpenic compounds and the antileishmanial, antioxidant and cytotoxic activities of *S. cilicica*, which is endemic and is utilized in traditional medicine (3, 4). Because the successful study results of the biological activities of *S. cilicica*, further studies on the species other possible usages shall be conducted. The present study concentrated on the compounds isolation and structure identifications (using spectroscopic methods ^{1}H NMR, ^{13}C NMR, HETCOR, COSY, NOESY, HSQC, HMBC, UV, IR, Mass) from the aerial parts and to determine the antimicrobial activity and preliminary studies on antiepileptic activity of the various extracts from the aerial parts and roots of *S. cilicica*. Keywords: *Salvia cilicica*, isolation, antimicrobial activity, antiepileptic activity **References:** [1] Davis P.H. (Ed.). Flora of Turkey and the East Aegean Islands. Vol VII. Edinburgh: Edinburgh University Press, 1988. [2] Davis P.H. (Ed.). Flora of Turkey and the East Aegean Islands. Vol X. Edinburgh: Edinburgh University Press, 1988. [3] Tan N. et al. Abietane diterpenoids and triterpenoic acids from *Salvia cilicica* and their antileishmanial activities. Phytochemistry. 2002; 61: 881 – 884. [4] Şen B., Yılmaz Özden T., Meriçli F., Meriçli A.H., Tan N., "Toros Dağları'nın Endemik Değeri *Salvia cilicica* Bitkisinin Aktivite Açısından Değerlendirilmesi", XX.BİHAT 10 – 13 September 2012, Antalya – Turkey

PJ48 Deconjugation of polyphenol-glucuronides by a factor with β-glucuronidase activity from human monocytes (Mono Mac 6 cells)

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Plant polyphenols are present in the human body predominantly as their phase II metabolites. Recent results demonstrate that β-glucuronidase from human neutrophils is able to deconjugate and thus activate glucuronide conjugates during inflammation (*in vitro*). In the present study, the ability of Mono Mac 6 cells (human acute monocytic leukemia cells) to deconjugate polyphenol-glucuronides was investigated. Baicalin (5,6,7-trihydroxyflavone-7-O-glucuronide (**1**) and 4-methylumbelliferone-glucuronide (**2**) were selected as test substances. The cells were seeded in 24-well plates (0.3×10^6 cells/ml) and grown for 48 h. After removing cells by centrifugation, the supernatant's pH was adjusted to 4 – 5 and was incubated with the substances (50 μM) over a period of 2, 4, 6, and 8 hours. The reaction was stopped by precipitating the proteins with MeOH, and the samples were analyzed by HPLC-DAD. The same procedure was performed without cells to ensure the stability of tested substances. Both glucuronides were deconjugated to a different extent: while 71% of **1** were deconjugated after 8 hours, only 53% of **2** were cleaved. The addition of TNF-alpha (110 U/ml) had no influence on the deconjugation, but the specific β-glucuronidase inhibitor D-saccharic acid-1,4-lactone (10 μM) completely inhibited this effect. No deconjugation could be observed at pH = 7.4. These results indicate the presence of a factor with β-glucuronidase activity, which seems to be secreted constitutively by the Mono Mac 6 cells and shows pH dependency. The varying extent of deconjugation is presumably based on different substrate structure.

PJ49 New approach to large scale isolation of rare prenylflavonoids

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Prenylflavonoids are an interesting group of natural products with different biological effects dependent on substitution pattern. Recently we showed that certain prenylflavonoids from hops boost the formation of neurons from stem cells (1). By a structure-activity study it was revealed, that pyranoflavonoids potently induce neurogenesis and the pyrano ring is one structural characteristic of this effect. Such molecules are present in hops to a very low extent. Nevertheless, from industrial scale hop extracts great amounts could be obtained, provided that a group separation method would be available. In this study we developed a strategy to separate groups of well known prenylflavonoids from neurogenesis-inducing pyranoflavonoids. Therefore we tested different stationary and mobile phases with exceptional selectivity to get a simple filtration method. Cyclodextrins were suitable due to their ability to differentiate between substances by forming inclusion complexes with more or less fitting guest-molecules. Using cyclodextrines as additives to the mobile phase and using a stationary phase with good binding characteristics to flavonoids, a very simple and quick method for separation was developed. Interestingly the separation is performed more as a filtration than as chromatography (2), making it possibly applicable for industry. Such extracts can be perhaps used in phytopharmaceutics regarding diseases like Alzheimer's, Parkinson's or even stroke or depression. **References:** [1] Aigner L., Riepl H., Urmann C., 2012 WO2012172090 A1 [2] Riepl H., Urmann C., 2012, Patent application DE10 2012 105 613.7

PJ50 Immunomodulating effects of *Biophytum petersianum*, a Malian medicinal plant

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Biophytum petersianum is an herb with a wide distribution in tropical countries. Field studies carried out in Mali show extensive use against cerebral malaria in children. It is used against pain and as a wound healing agent, as well. The aim of this study was to investigate the immunomodulating effects of extracts and flavonoids isolated from *B. petersianum*. Aerial parts of *B. petersianum* were extracted with dichloromethane followed by methanol. The methanol extract was further partitioned between ethyl acetate, butanol and water. The ethyl acetate extract was fractionated by flash chromatography and preparative HPLC, and chemical structures of isolated compounds were elucidated by NMR spectroscopy. The effect on NO secretion from macrophages and complement fixing activity were used to evaluate the immunomodulating activities. Three flavonoids were isolated from the ethyl acetate extract; cassiaoccidentalin A, isoorientin and isovitexin. The crude methanolic extract was a good inhibitor of NO secretion and it showed strong effect in the complement fixation test. The immunomodulating effects were potentiated in the ethyl acetate extract, thus indicating that the bioactive compounds are medium lipophilic. However, the flavonoids isolated in this study were either inactive or they showed only moderate effects in the bioassays. It appears that so far unidentified bioactive substances are present in the ethyl acetate extract and that further research is needed to identify immunomodulators in *B. petersianum*.

PJ51 Traditional Japanese "Maccha" type green tea (*Camellia sinensis* (L.) Kuntze) from Uji for metabolic syndrome therapy: An open-label clinical pilot study

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Green tea is a classical cultural asset of Japan, with Maccha (抹茶) – extremely fine powdered dried leaves that are drunk as a suspension in hot water – being one of its most refined forms. It is today consumed worldwide for its general health effects which are mainly attributed to its prominent phenolics, e. g. epigallocatechin gallate. Its alleged activity against the metabolic syndrome and obesity was met with a lot of attention in recent years as their spread in the industrialised world has become a serious public health concern. In the present study, 10 volunteers (7 male, 3 female) with metabolic syndrome took Maccha from the traditional Japanese tea cultivation centre in Uji near Kyoto which was formed into tablets of 1 g each. Treatment consisted of 3 times 5 tablets, corresponding to 15 g of Maccha every day during a time frame of 60 days. Before and after the trial, waist circumference, body weight, blood pressure, and the amount of body fat were measured together with physiological parameters from the biochemical analysis of blood and urine samples. At the end of therapy, low-density lipoprotein (LDP) ($p = 0.026$), alkaline phosphatase (ALP) ($p = 0.035$), lactate dehydrogenate (LDH) ($p < 0.001$), total protein (TP) ($p = 0.007$), globulin ($p = 0.021$), and platelets ($p = 0.002$) were increased, while mean corpuscular haemoglobin (MCH) ($p = 0.001$) was decreased throughout the patient collective. Five of the male patients lost weight during the treatment. In this population, the physiological values for ALP ($p = 0.011$), LDH ($p = 0.012$), TP ($p = 0.034$), globulin ($p = 0.022$), and platelets ($p = 0.002$) were significantly increased, whereas those for uric acid (UA) ($p = 0.022$), albumin/globulin (A/G) ratio ($p = 0.025$), and MCH ($p = 0.001$) were decreased. In consequence, long term intake of Maccha can improve TP, globulin, and cholesterol values, thus enhancing energy and fat metabolism, important for the prevention of arteriosclerosis.

PJ52 Quantification of isoorientin in *Passiflora edulis* rinds by HPTLC-densitometry and HPLC/DAD methods and evaluation of radical scavenging capacity of the extracts

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Passiflora edulis fo. *flavicarpa* O. Degener (Passifloraceae) is widely cultivated in Brazil mainly for industrial juice production and is also consumed in natura. Passion fruit rinds represent about half of the fruit's mass, and are a major waste product. The purpose of this study was to develop analytical methods based on HPTLC-densitometry and HPLC/DAD for the determination of isoorientin in *P. edulis* rinds. Preliminary tests indicated isoorientin as the major flavonoid and the only relevant compound of the rinds with availability of commercial analytical standards. In addition, the antioxidant capacity (radical scavenging) of passion fruit extracts was evaluated by the DPPH· (2,2-diphenyl-1-picrylhydrazyl radical) method, in view of the potential use of passion fruit rind by-products. The content of isoorientin in rinds found by HPTLC ($0.206 \pm 0{,}001$ mmol L^{-1}) was higher than the result obtained by HPLC/DAD ($0.101 \pm 0{,}001$ mmol L^{-1}). This discrepancy may be explained by the possible overlapping bands (compounds) or also due to the matrix effect in HPTLC. The total volume of organic solvent employed in the HPTLC method was eight times lower compared to the HPLC/DAD method, and the duration of HPTLC analyses was six times shorter than the HPLC/DAD method, indicating the potential of the former as a "green" alternative technique to HPLC in routine qualitative and quantitative analyses of *P. edulis* foods and extracts, with smaller consumption of solvents and reagents. Besides, the antioxidant capacity of passion fruit rinds ($EC50 = 25.93 \pm 1.80$ g L^{-1}) was found to be considerably higher than in other foods such as sugarcane juice ($EC50 = 100.80 \pm 2.56$ g L^{-1}) and passion fruit pulp ($EC50 = 38.50 \pm 2.28$ g L^{-1}), suggesting a direct correlation between antioxidant capacity and isoorientin content in *P. edulis* rinds, according with previous studies. These results suggest the relevance of in-depth research into passion fruit rind as a potential source of natural antioxidants.

PJ53 Isolation of caffeic acid derivatives and their anti-inflammatory effects in raw 264.7 cells from *Parasenecio firmus*

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It is well known that the pro-inflammatory mediators such as nitric oxide (NO) and prostaglandin E_2 (PGE_2) are involved in several inflammatory diseases and lipopolysaccharide (LPS) can stimulate these inflammatory responses. Caffeic acid derivatives (CAD) are treated as important anti-inflammatory chemicals. The aim of this study is to investigate isolation and anti-inflammatory effects of CAD which are the major compounds of *Parasenecio firmus*. Bioassay-guided separation on methanol extract of *P. firmus* using the multiple chromatography steps resulted in the isolation of three CAD that are identified as 3,5-caffeoylquinic acid, 4,5-dicaffeoylquinic acid and 5-caffeoylquinic acid (5-CQA). The anti-inflammatory effects of CAD were investigated *via* study on the molecular mechanisms in lipopolysaccaride (LPS)-stimulated RAW 264.7 cells. CAD dose-dependently reduced the productions of NO and PGE_2 induced by LPS. It was found that 5-CQA showed the highest inhibition on NO production in RAW 264.7 cells. In addition, 5-CQA significantly suppressed the LPS induced expressions of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and tumor necrosis factor-α (TNF-α) at the mRNA and protein levels. These results were strongly supported by decrease in DNA binding of nuclear factor-kappa B (NF-κB) and nuclear translocation of p65 and p50 subunits of NF-κB. in addition, 5-CQA dose-dependently reduced the production of IL-6, IL-1β and TNF-α in LPS-stimulated RAW 264.7 macrophage cells. Thus, these results suggest that the anti-inflammatory activity of 5-CQA is associated with the down-regulation of iNOS, COX-2 and TNF-α through the negative regulation of the NF-κB pathway in RAW 264.7 cells.

PJ54 Flavonoids and caffeoylquinic acids from *Solanum paniculatum* L. (Solanaceae)

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Solanum paniculatum, is a shrub up to 3 feet tall, with short, curved spines and its fruits are greenish-white containing numerous lenticular seeds. Their roots, stems, leaves and fruits are used in folk medicine as well as in culinary.[1] It was formerly included in Brazilian Pharmacopoea, but was removed from newer editions. In 2006, Brazilian Ministry of Health published a list, comprising 71 species, including *S. paniculatum* to guide research, aiming at the expansion of the number of herbal medicines available for basic pharmaceutical care in Brazil. The positive results found when water extracts of *S. paniculatum* were tested for their antiulcer and gastric activities validate folk use of *S. paniculatum* against gastric disorders.[2] Recent reports[3] support the use of *S. paniculatum* preparations for hepatoprotection. Our report focus on the results of the phytochemical analysis of the ethyl acetate partition, obtained from the water extract of leaves of *S. paniculatum.* Chlorogenic acid was found as the main compound in the extract, together with di- and tricaffeoylquinic acids. Four flavonoids were also identified: isoquercetrin, a hexoside of kaempferol and two glycosides of quercetin. The presence of caffeoylquinic acids and flavonoids may be associated to the reported hepatoprotective activity of *S. paniculatum* extracts. We are now evaluating the potential antioxidant properties of ethyl acetate partition ex-

tracts of *S. paniculatum* and its *in vivo* hepatoprotective activity against paracetamol-induced hepatotoxicity. **Acknowledgements:** CNPq, FAPERJ and CAPES **References:** [1] Mors, W.B.; Rizzini, C.T.; Pereira, N.A. Medicinal Plants of Brazil, Ed. Robert A. DeFilipps, Reference Publications Inc., 2000. [2] Mesia-Vela, S.; Santos, M.T.; Souccar, C.; Lima-Landman, M.T. and Lapa A.J., Phytomedicine 9:508 – 14 (2002). [3] S.M. Sabir, S.M. and Rocha, J.B.T. Journal of Ethnopharmacology 120: 226 – 232 (2008)

PJ55 Determination of phenolic compounds in *Pinus eldarica* by HPLC

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The antioxidant components have been identified in some pine species. Antioxidant properties of proantocyanidins reduce free radicals induced by DNA fragmentation and lipid proxidation and also proanthocyanidines could curb lipid peroxidation. In this study, we analyzed different parts of *Pinus eldarica* (bark, seed and needle) and assessed their antioxidant contents. The HPLC method (UV detector, C_{18} reverse phase column, 4.6 mm' 25 cm, and water/H_3PO_4/methanol/acetonitril as eluant) was employed for evaluating total polyphenols. The highest range of total polyphenols was detected in the bark of this pine. The high amount of total phenolic compounds in P. eldarica bark might be attractive for future research considering its health benefits.

PJ56 Phenolic Compounds and Antimicrobial Activity of *Inula sarana* Boiss.

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The genus *Inula* (Asteraceae) has more than hundred species distributed mainly in Europe, Africa and Asia. *Inula* species are used as a folk medicine due to their anti-inflammatory, antiseptic, antipyretic, hepatoprotective, antitussive and expectorant effects (1). *Inula sarana* Boiss. is an endemic herb growing wild in southern Turkey (2). Up to now, only one study on this plant has been conducted in Turkey (3). In the coarse of our continuing search on the *Inula* species, we investigated the total phenolic content, phenolic compounds and the antimicrobial activity of different parts of *Inula sarana*. Total phenolic content of the plant was estimated using the Folin-Ciocalteu method. The higher amount of total phenolics was found in the aerial parts of the plant. The qualitative and quantitative analysis of the phenolic compounds were performed by RP-HPLC. Our results revealed that while all the investigated parts of the plant contain chlorogenic and ferulic acids; the flowers and leaves also contain gallic acid and luteolin; apigenin was determined only in the flowers. Antimicrobial activity of the flower, leaf and root extracts of the plant were assayed against *S. aureus*, *E. faecalis*, *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris*, *E. aerogenes*, *E. coli*, *C. albicans* and *C. tropicalis* by using agar dilution method. The flower and root extracts were found to be more active against yeasts compared to bacteria. The flower extract exhibited the most effective antibacterial activity against *S. aureus* and *K. pneumoniae*, while the leaf extract is very potent against *S. aureus* and *E. coli*. **References:** [1] Zhao, Y-M. et al. (2006) *Chem. Biodivers*., 3:371 – 384. [2] Davis, P.H. (1982) Flora of the Turkey and The East Aegean Islands, Vol 5, University Press, Edinburgh. [3] Kirimer, N. et al. (2009) *Planta Med*., 75(4):421.

PJ57 Polyphenolic composition and antichlamydial effect of commercial peppermint (*Mentha* x *piperita* L.) teas

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The qualitative and quantitative polyphenolic content of the infusions of the commercial peppermint tea (*Mentha* x *piperita* L.) samples (n = 27) from different countries was studied using HPLC-UV-MS/MS analysis. Overall, 22 polyphenols were identified in the peppermint infusions. The major polyphenols were eriocitrin, 12-hydroxyjasmonate sulphate, luteolin-*O*-rutinoside and rosmarinic acid. The total polyphenolic content varied largely among the 27 peppermint tea infusions, found in a range of 10.0 – 218.0 mg/ml. In order to determine the content of samples by finding chemosystematic markers, essential oil composition of the samples was determined by GC. Of the analysed peppermint tea samples, 24 met the standards set by Ph. Eur. 7th Ed., whereas the analyses indicated that three samples may contain *Mentha spicata*, a species different from that claimed on the package. The effects of seven peppermint tea extracts against a respiratory tract pathogen *Chlamydia pneumoniae* were investigated *in vitro*. All the teas prepared from the selected commercial peppermint products inhibited chlamydial growth, inhibitions ranging from 20.7 to 69.5% at the extract concentration of 250 μg/ml. The effect on the inclusion counts at the second passage of infection was studied, showing an inhibitory effect on the infectious progeny production ranging from 7.8 to 78.1%. In most cases, the antichlamydial activity was a characteristic of the peppermint teas having high contents of luteolin and apigenin glycosides. This study supports the consumption of peppermint tea to potentially elicit beneficial health effects on acute respiratory tract infections.

K. Quality control methods for medicinal plants, extracts and isolated natural products

PK1 Evaluation of the *in vivo* antimalarial potentials of the leaf and fruit of *Uvaria chamae* P. Beauv (Annonaceae)

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Ethnomedicinally, the leaves and fruits of *Uvaria chamae* P. Beauv (Annonaceae) are used in traditional preparations for febrile illness. Therefore, their antimalarial activities were evaluated with a view to justifying this ethnomedical use. The root has been scientifically proven to have antimalarial activity [1]. Methanolic extracts of the dried leaves and fresh fruits administered at 100 – 800 mg/kg on *Plasmodium berghei*-infected mice were evaluated using the four-day (chemosuppressive) and curative (Rane's) antimalarial test models; distilled water and amodiaquine (10 mg/kg) were negative and positive controls, respectively. At 800 mg/kg, leaf and fruit extracts gave chemosuppression of 42 and 28% (four-day test) and parasite clearance of 36.3 and 49.5% on day 5 (curative test), respectively while the positive control-treated groups were 72.8% and 98%. The mean survival times were comparable ($p > 0.05$) to the amodiaquine-treated group in both leaf- ($P = 0.83$) and fruit- ($P = 0.30$) treated mice in the chemosuppressive test but significantly lower for the leaf- ($P = 0.006$) and fruit- treated ($P = 0.002$) groups than that of the amodiaquine- treated group (24 days) in the curative test. No toxic effects were observed at the doses used. The leaf and fruit extracts showed better chemosuppressive and curative antimalarial activity, respectively thus justifying their folkloric uses. **References:** [1] Okokon, J.E., Ita, B.N. and Udokpoh, A.E. (2006). The in vivo antimalarial activities of *Uvaria chamae* and *Hippocratea Africana* Annals of Tropical Medicine & Parasitology 100(7) 585 – 590.

PK2 Pharmacognostic studies and establishment of quality standard for leaves of *Harungana madagascariensis* (Choisy) Poir (Clusiaceae)

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Introduction; *Harungana madagascariensis*, (Choisy) Poir Family Clusiaceae is a small to medium tropical shrub (up to 1.65 m high) with fine stellate hairs and ovate lateral leaves used in European herbal medicine to treat indigestion and poor pancreatic function and in African folk medicine as a treatment for diarrhoea and dysentery. The sap is used in the treatment of scabies and as an anti-helmintic (tapeworms), while leaves are used as a remedy for hemorrhages, diarrhea, gonorrhea, sore throats, headaches and fevers. Resin from the flower stalks is believed to ease colic and to check infection after childbirth, while a decoction of the bark is drunk as a remedy for malaria. Young leaves are sometimes used as medicine for asthma and fruits are occasionally used in cases of abortion in the belief that the red juice averts bleeding.(Keay 1989) **Methods** Pharmacognostic studies were carried out using the World Health Organization (WHO) Ouality control methods for medicinal plants (1998) **Results;** Transverse section through the mid rib of the leaf shows the presence of a single layered, thick wall epidermal cells 4 – 6 sided covered with hairs on both the adaxial and abaxal surfaces. The leaf is hypostomatic and the stomata type is diacytic. Powdered microscopy of the leaf shows diagnostic features as glandular trichomes with many stellate trichomes, calcium oxalate crystals, sclerids and fibers. Physico-chemical parameters determined included extractable matter both for hot water (62.13 mg/g) and cold water (46.75 mg/g) as well as hot alcohol (82.63 mg/g) and cold alcohol (82.63 mg/g.) **Conclusion;** The results obtained from the study can serve as the basis for the preparation of monograph for the plant and subsequent incorporation into Nigeria Herbal Phamacopea **Reference:** [1] Keay RW. 1989. Trees of Nigeria. Claredon Press Oxford. [2] WHO (1998) Quality control methods for medicinal plants

PK3 Introducing green technology for extraction of medicinal and aromatic plants to Egypt

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With the increasing demand for herbal medicinal products, nutraceuticals, and natural products for health care all over the world, medicinal plant extract manufacturers and essential oil producers all over the world have started using the most appropriate extraction technologies in order to produce extracts and essential oils of defined quality with the least variations from batch to batch. It has become absolutely necessary to disseminate such innovated technology to emerging and developing countries for the best industrial utilization. Microwave and Ultrasound assisted extractions have been recently introduced to Phytochemistry Department, National Research Centre, Egypt. Optimization of extraction methods of economically important medicinal plants such as *Cynara scolymus* and *Silybum marianum* was carried out. HPLC demonstrated that total silymarin content extracted from *Silybum marianum* L. has been increased by using microwaves compared with traditional extraction method (0.43, 0.36%; respectively) with higher Silybinin A and Silybinin B contents (8.7, 2.3% for silibinin A and 16.6, 5.2% for silibinin B). For *Cynara scolymus* L., the total extraction yields were 17, 30 and 20% for traditional, microwave and ultrasonication methods; respectively. Chlorogenic acid content was determined by RP-HPLC. Much higher yield of chlorogenic acid was obtained by using microwaves and ultrasound assisted extraction compared with the classical method (50.607, 50.761 and 18.020%; respectively). **Acknowledgement:** This work is a part of a project financially supported by Science and Technology Development Fund (STDF) **References:** [1] Extraction Technologies for Medicinal and Aromatic Plants United Nations Industrial Development Organization and the International Centre for Science and High Technology, **2008.** [2] Saleh N. A. M., Global Phytochemistry: the Egyptian Experience, Phytochemistry, 63, 239 – 241, 2003.

PK4 The quantitative analysis of curcuminoids in a food additive and foods evaluated using rapid HPLC with electrochemical, UV or fluorescence detection

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Turmeric, the rhizomatous herbaceous perennial plant of the ginger family, in dried powdered form is used as a culinary additive to impart a distinctive yellow-orange color to Pakistani, Indian and Thai cuisines. Although turmeric has been used for many years in Ayurvedic medicine, its potential use in Western medicine has only recently been explored. Research to date suggests that turmeric, besides having an immunomodulatory role, is also of use in preventing oxidative stress that can lead to inflammation, cancer and arthritis. The natural products in turmeric that are purported to possess health benefits include a number of curcuminoids including curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). A rapid HPLC method using a Thermo Fisher Scientific Dionex UltiMate 3000 electrochemical detector for the simultaneous measurement of C, DMC and BDMC was developed. Curcuminoids in three food products, including turmeric power, curry powder and a pellet curry sauce, were quantitatively determined after simple ultrasonic extraction with methanol. Separation of curcuminoids was achieved in just 3 min using a Thermo Fisher Scientific Dionex PA2 column (2.2 µm particles, 2.1 x 50 mm). With electrochemical detector, the LOD (S/N = 3) was 2pg (on column) for C and DMC and 4pg for BDMC; intraday precision (n = 10) was < 10% for 10pg (on column) and < 4% for 20pg curcuminoids. A comparison of different detection technologies showed that ECD was more sensitive than UV detection, and was both more sensitive and had more uniform response than fluorescence detection.

PK5 Novel, universal approach for the measurement of natural products in a variety of botanicals and supplements

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Botanicals contain a great diversity of compounds that exhibit wide variation in their physicochemical properties. Although no single analytical method is available to measure all potentially active components, HPLC with charged aerosol detection is a nearly universal approach that nively measures any nonvolatile and many semivolatile compounds; that is, CAD does not require that analytes be ionizable (as required for mass spectrometry) or contain a chromophore (as required for UV spectrophotometry). A number of isocratic and gradient HPLC/UHPLC methods with charged aerosol detection were developed and evaluated for the measurement of phytochemicals extracted from a variety of botanicals including: steroidal and pregnane glycosides from *Hoodia gordonii;* oxypregnane glycosides from *Caralluma fimbriata;* steroidal lactones from *Withania somnifera;* flavonolignans from milk thistle (*Silybum marianum*); triterpene glycosides from black cohosh (*Cimicifuga racemosa*); ginsenosides from ginseng (*Panax ginseng*); and diterpene glycosides from stevia (*Stevia rebaudiana*). Analytes showed consistent response independent of chemical structure (typically < 10% variability between compounds corrected for gradient elution). All methods had a wide dynamic range (~four orders of magnitude), good sensitivity (typically low ng levels of detection), and excellent reproducibility (RSDs typically < 2%) even at low detection levels. Comparative data from ELSD and UV detection will also be discussed.

PK6 Evaluation of herb and fruit juice adulteration and authenticity by coulometric array detection and pattern recognition analysis

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Although the adulteration of herbs and fruit juice is a frequent phenomenon, there are few simple methods available for the screening of large numbers of commercial batches of product. The challenge arises from

the complexity and variability of genuine material combined with the unrelenting conduct of adulteration. Herb and fruit variety, growing region, season, ripeness, and processing methods all contribute to the variability of the authentic product, making unambiguous characterization difficult. Currently, the most reliable and applicable authentication methods are based on analytical chemical fingerprinting (metabolomic) techniques. HPLC with coulometric detection is particularly suitable for generating chemical fingerprint for herb and fruit juices that contains endogenous electrocitive components essential to their flavor, stability, color and aroma. An extension of phytochemical fingerprinting utilize statistical program such as principal-component analysis and pattern recognition to evaluate the authenticity of a given sample by comparing its chromatogram with a compiled population of authenticated reference sample. We investigated the approach of using HPLC-Coulometric Array detection with pattern recognition software to identify potential oregano herb and fruit juice adulteration. Oregano adulteration by blending with adulterants such as thyme and marjoram, were readily detected. In a second study, fruit juice adulteration using dilutions with another juice, or the inclusion of orange peel/pulp-wash could readily be evaluated using this technique. The specificity of this approach even allows the classification of orange juice samples by varietal and geographical region.

PK7 A new validated HPLC-DAD method for the simultaneous determination of six phenylethanoid glycosides in two *Scutellaria* species

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The genus *Scutellaria* L. which belongs to the family Lamiaceae, encompasses about 350 species [1]. 16 species of *Scutellaria* grow wild in the flora of Turkey [2]. Antitumor, antiangiogenic, hepatoprotective, antioxidant, anticonvulsant and neuroprotective effects of several *Scutellaria* species have been reported. The chemical composition of *Scutellaria* species have also been studied since 1889 and over 295 phytoconstituents have been isolated [1]. Although many HPLC studies have been published reporting the determination of several flavonoids [3], there is no HPLC work focusing on the phenlyethanoid profile of the *Scutellaria* species. In this study we have developed a simple, sensitive, precise, accurate and a validated reversed phase high performance liquid chromatographic method for the simultaneous determination of six phenylethanoid glycosides (calceolarioside D, neocalceolarioside D, verbascoside, isoverbascoside, leucoseptoside A, martinoside) in MeOH extract of the aerial parts of *S. hastifolia* and *S. velenovskyi*. The method involved the use of Zorbax® XDB C_{18} column (4.6 x 150 mm, 3.5 µm) at 25 °C with the mixture of MeCN and H_2O gradient (15 – 45 % MeCN in 35 min.), constant flow rate 0.8 ml/min) as a mobile phase and detection at 330 nm. Calibration plots were linear in the range of 3 µg/mL-120 µg/mL for calceolarioside D, neocalceolarioside D, verbascoside, isoverbascoside and martinoside, 1 µg/mL-120 µg/mL for leucoseptoside A. The amounts of above phytoconstituents varied among the species. This method can be used for the determination of phenylethanoid glycosides which constitute one of the major chemical classess of the genus *Scutellaria*. **References:** [1] Shang et al., Journal of Ethnopharmacology. 128 (2010):279 – 313. [2] Edmonson J.R. *Scutellaria* L. In "Flora of Turkey and East Aegan Islands" Davis P.H. (ed.) Edinburgh University Press. Edinburgh. pp. 78 – 100 (1982). [3] Gao et al., Journal of Pharmacy and Pharmaceutical Sciences, 11 (2008): 77 – 87.

PK8 Development of an HPLC method for quality control of *Alpinia zerumbet*

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The Brazilian National Medicinal Plants and Herbal Medicines Programme provides for the distribution of herbal medicines in all local municipal, state and federal health services. *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M.Sm. is traditionally employed to treat hypertension. The Herbal Medicines Formulary of the Brazilian Pharmacopoeia lists *Alpinia zerumbet* as a diuretic and for the treatment of mild hypertension. However no chromatographic method for quality control is described. Since flavonoids are involved in the pharmacological activity, a HPLC method has been developed for the analysis of *Alpinia zerumbet* aqueous extract, and Rutin was selected as reference for quantification. Using statistical experimental design the method was optimized as regards the amounts of acetonitrile, acidified water and methanol in the mobile phase composition using an RP-18 column. All analyses were performed with aqueous *Alpinia zerumbet* extracts. In five runs an optimal separation was approached. A linear regression of rutin was performed and with the optimized mobile phase composition it was possible to analyze the flavonoid glycosides present in the extract. Regression analysis indicated an adequate fit with a determination coefficient (R^2) of 0.9999. Repeatability was also adequate with maximum relative standard deviation of 1.84%. The rutin content was calculated as 0.44% and the total amount of flavonoid glycosides, calculated by rutin regression, was estimated to be 4%. This developed procedure is important for the systematic quality control of the herbal drugs and their preparations in ensuring the degree of safety, efficacy and quality required by the National Health Programme.

PK9 Linking analysis to value chains: Using NMR spectroscopy and HPTLC to analyse the metabolite variability of turmeric products

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Introduction: It is well known that raw materials are vital components of herbal products and while considerable attention has been paid to assessing the pharmaceutical quality of the final product, surprisingly little is known about the variability of the starting material and about the value chains from producer to consumer (Booker et al 2012). Stringent procedures are needed for their cultivation and primary processing if the finished product is to approach high level of quality. Research often focuses on developing improved varieties but not on the variability of the starting material sourced from multiple producers. Objectives: To determine the variability of metabolite content in a range of turmeric samples collected in India and in commercially available products and assess the strengths and weaknesses of two selected analytical techniques. Leading on from this primary goal our secondary objective was to attempt to link variability in product composition to the methods of production and the value chain. Methodology: Fifty samples of turmeric products were gathered from sites in India, Europe and the USA. The samples were analysed using proton-NMR linked to multi-variate analysis software to identify differences in the metabolite content. The samples were subsequently analysed using HPTLC to discover if this technique gave similar information to NMR and if there were any benefits gained by employing a dual analysis strategy. **Results:** It was found that samples varied in metabolite composition and the NMR-Multivariate analysis platform was able to group samples according to these differences. HPTLC generally gave similar results but with some notable exceptions which gave rise to further investigation. (Fig 1)

PK10 A comparative study on *Pyeongwi-san* using different extraction conditions

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Decoction, in Korean Medicine, is a pharmacological method of extraction, by boiling, of dissolved chemicals, or herbal prescriptions, which may include stems, roots, barks and rhizomes. Decoction differ from most teas, infusions, in that they are usually boiled. This study was performed to compare the difference of decoctions extracted by different decoction extractor and extraction time for standardization of extracting method. *Pyeongwi-san*, an herbal prescription, consists of Atractylodis rhizoma, Citri pericarpium, Magnoliae cortex, Glycyrrhizae radix,

Zingiberis rhizoma and Ziziphi fructus. *Pyeongwi-san* extracts were investigated the yield and the concentration of hesperidin and glycyrrhizin and anti-inflammatory effect. The analyzed patterns of HPLC were similar. Among the extractors, ultrasonic wave merged extractor showed the highest yield and concentration of hesperidin and glycyrrhizin when extracted for 1 *hr,* 2 *hr* and 3 *hr.*

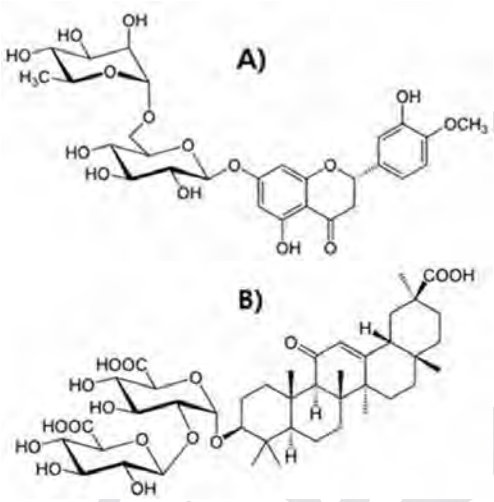

Fig. 1: The structures of hesperidin (A), and glycyrrhizin (B).

Samples extracted for 2 *hr* using three types of extractor were investigated on anti-inflammatory effect in peritoneal macrophages, and 10 μg/mL, 100 μg/mL, 1,000 μg/mL concentration of samples were used in this assay. In nitric oxide (NO) assay, NO inhibition were significant in 100 μg/mL concentration sample which was extracted using pressured extractor. Statistical analysis using Tukey test showed significance in all groups except for the 10 μg/mL of Non-pressured extractor. The data rank of NO assay differed from the data rank of the HPLC and the reason was considered to be affected by other possible variables. Above results showed that the pharmacological effects of decoction could be changed by altering method and extractor. And it was still impossible to determine decoction method by several characteristics such as the yield and concentration of reference compounds.

PK11 Simultaneous qualitative and quantitative analysis of phenanthrene derivatives in *Vanda coerulea* by LC-HRMS/MS

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Previous phytochemical analyses of crude extracts of *Vanda coerulea* Griff ex. Lindl (Orchidaceae) have led to the identification of stem-specific metabolites. In total, one hydroxy-benzyl acid and 5 chemically diverse phenanthrene derivatives were identified, including one bibenzyl, two phenanthropyrans and two dihydrophenanthrenes. These six compounds were identified for the first time together in one species and are considered as *V. coerulea* chemical markers. The three most concentrated compounds (imbricatin, methoxycoelonin and gigantol) displayed antioxidant and skin anti-inflammatory activities and can thus be considered also as biomarkers [1]. Different studies have been led to identify in which vegetative stage (plants with bud, before or during flowering) these metabolites are the most concentrated. The quantitative analysis was performed by a LC-HRMS/MS developed and validated method. For practical reasons, this study was conducted on a population of *V. coerulea* cultivated in a green house. Notable differences were observed among the different vegetative stages. In February, they are concentrated in flowering plants then their amount increase in October. In every case, *V. coerulea* specimens in bud contain the least metabolites of interest. The developed method permitted to better control a cultivated population of orchids in an artificial environment. If our results are confirmed on wild population growing in a natural biotope, we can determine in the future the optimal conditions of collecting the high-value added plant material for industrial valorization. **References:**

[1] Simmler C., Antheaume C., Lobstein A.: *PlosOne*. 2010 Oct 28;5(10):e13713.

PK12 The ash content in dried fruits of *Vitex agnus-castus* – wild collecting vs. controlled cultivation

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Ash contents take an important part in the evaluation of raw materials for pharmaceutical use. In the monograph of the Pharm. Eur the ash content for dried fruits of *Vitex agnus-castus* is limited at max. 5.0%. Until now the procurement of *Vitex agnus-castus* resulted predominantly from wild collection. Generally this limit wasn't exceeded as far as the harvest and the postharvest treatment were carried out properly and under submission of GACP guidelines. In the controlled cultivation the situation is different. The ash content maximally permitted frequently is exceeded by 5.0% (up to 8.0%). In order to explain the increase of ash contents, detailed investigations delivered no variation of the ash contents after different washings of the fruits of Vitex. In further experiments, the mineral composition of dried Vitex fruits of wild collection and of controlled cultivation was analyzed. Increased contents were found mainly in the macro and micro elements [potassium, calcium, phosphorus, magnesium, iron and manganese]. Oppositely, no increase was measured in elements, which according to the current knowledge are not essential for the nutrition of higher plants, as cadmium, lead, silver and cobalt. The values determined in the fruits of Vitex deriving from cultivation do not differ considerably from the fruits contents of various other plant species. In summary, the high content of ash determined in the dried fruits of Vitex produced under controlled cultivation in comparison with fruits out of wild collection isn't due to soiling during harvesting or the steps after. The higher concentration of the mineral components is based on the use of fertilizers in the controlled cultivation. However, the nutrient contents in the fruits of Vitex are in a normal range in the plant kingdom. Due the optimal provision of the plants with fertilizers a rise of the nutrient contents in different plant parts and thus the entire ash content of the fruits is to expect.

PK13 Quality control in the production of microencapsulated natural antioxidants from Clove (*Syzygium aromaticum*)

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In the present work, powdered lipid formulations encapsulating natural antioxidants from Clove (*Syzygium aromaticum*) were prepared by spray drying and freeze drying. The microencapsulation of eugenol (EU) and eugenyl acetate (EA), the main phenolics compounds from clove, is justified by their volatility and poor water solubility. Lipid formulations have gain especial attention as delivery systems of plant-derived compounds due to their encapsulation efficiency, stability and modification of solubility. The formulation developed in this work was composed by a mixture of the solid lipid glyceryl behenate (Compritol 888 ATO), the liquid lipid buriti oil (*Mauritia flexuosa),* a surfactant Poloxamer 188 (Kolliphor® P 188) and Maltodextrin DE10 as drying carrier. High Performance Liquid Chromatography (HPLC) was employed to monitor the concentration of EU and EA during the production process of the microcapsules, which involves the extraction process, preparation of the liquid formulation involving homogenization by ultrassound and the drying process. Results showed that the concentration of EU and EA was significantly higher in the powder obtained by freeze drying than the one obtained by spray drying, and the antioxidant activity was correlated with the concentration of those compounds. The main advantage of freeze drying over spray drying is that the losses of compounds was significantly lower, although it is a more expensive and time consuming process.

PK14 Isolation of grayanotoxin I from *Rhododendron* species and quantification by densitometry

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Species of the family Ericaceae are a potential source of toxic diterpenes, named grayanotoxins (GT). Grayanotoxin I (GT-1, Fig.) is one of the main compounds also known as acetylandromedol or andromedotoxin. GT-1 causes a number of livestock poisoning and food intoxication by toxic honey ("mad honey") made by bees in the Black Sea region of Turkey. The symptoms of intoxication are related to the concentration and could cause a simple weakness up to cardiac problems. Because of missing data about the distribution of GT in different species of the Ericaceae, it is important to isolate pure GT as a reference standard to develop analytical methods for detection and to study the pure toxin. In this work we describe a way to isolate GT-1 from leaves of *Rhododendron* species which were deep frozen immediately after the harvest. The plant material was extracted with hot methanol, and the dried extract was partitioned between water and dichloromethane. Final purification was achieved by silica gel chromatography. Afterwards, we developed a densitometric method to quantifiy the amount of GT-1 in different *Rhododendron* species. Using a standard TLC plate we were able to analyse 11 samples simultaneously. To visualize the GT-1 we used 60% sulfuric acid and heated the plate for about 3 min at 100 °C. GT-1 was quantified by densitometry recording the remission of the TLC-spots at 530 nm. The quantification was based on an extern calibration considering the Kubelka-Munk function.

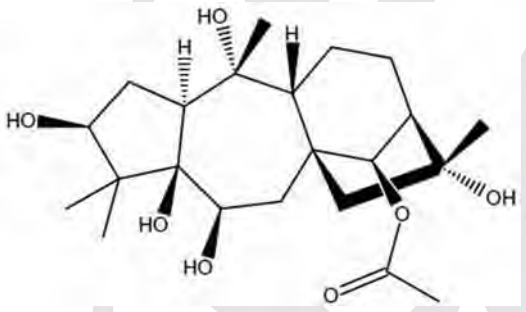

Fig. 1

References: [1] Humphreys DJ, Stodulski JBJ (1986) Detection of andromedotoxins for the diagnosis of *Rhododendron* poisoning in animals. Journal of applied toxicology, 6(2), 121. [2] Louis A, Petereit F, Lechtenberg M, Deters A, Hensel A (2010) Phytochemical characterization of *Rhododendron ferrugineum* and in vitro assessment of an aqueous extract on cell toxicity Planta Medica 76 (14) 1550. [3] Koca I, Koca AF (2007) Poisoning by mad honey: A brief review. Food Chem Toxicol. 45(8) 1315.

PK15 Stability Testing of Herbal Medicinal Products – Bridging Science to Industry

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Stability data for Herbal Medicinal Products (HMPs) are required prior to registration for both procedures, a full application based on well established use (WEU) or traditional use (THMPD, Directive 2001/83/EC). The aim of this lecture is to bridge scientific research with the legal requirements and industry's expectations:

1. general requirements for stability testing from GMP, ICH and their particular aspects concerning HMPs
2. analytical aspects such as choice of markers, choice of methods and shelf-life specifications

with special regard to herbal products. On this basic approach the presentation gives a review of the more general guidelines applied, e.g. Guideline on Stability Testing: Stability Testing of Existing Drug Substances and Related Finished Products, CPMP/QWP/122/02, Rev 1 corr, and the Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products, CPMP/QWP/2819/00, rev 1, currently reviewed by Questions & Answers on Quality of Herbal Medicinal Products/Traditional Herbal Medicinal Products, EMA/HMPC/41500/2010 rev. 1, 22 Nov 2011, including current requirements such as the WHO General Guidance for Inspectors on "Hold-Time" Studies, Working document QAS/13.521, published in February 2013. To look ahead, the talk will also consider the impacts of the drafted new monographs for Herbal Drug Extracts, issued in Pharmeuropa 25.2 (March 2013). The isolation and structure elucidation of new chemically defined substances in herbal plants, drugs and drug preparations are a helpful support not only for understanding the active principle of the HMPs but for analytical quality control purposes, too. Presenting practical examples from a industry's R&D and routine quality control (QC) laboratory it is shown which demands should be met to establish a powerful analytical marker for the daily QC-lab work as a guiding advice for scientific research.

PK16 Hydrolysis of *Helleborus niger* L. saponins for aglycone analysis

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Saponins are secondary metabolites in plants holding a defensive role. They are chemically split up into triterpene and steroid saponins. Generally, saponin structures may attain a great diversity due to various possible substituent patterns. They possess a wide range of pharmacological properties including cytotoxic and antitumor activities. Especially steroid saponins of the diosgenyl group showed remarkable effects [1]. Macranthogenin, a sapogenin similar to diosgenin, is the only aglycone described for *H. niger* L. so far [2]. Therefore additional research effort is needed to get a more complete view of its sapogenin composition. Due to the risk of artefact formation, saponin hydrolysis is the crucial step for aglycone analysis. Acid hydrolysis (1N HCl, 100 °C, 1 h, GC-MS) of the whole *H. niger* L. saponin fraction resulted in a complex peak mixture. Further experiments on isolated macranthosid I showed the introduction of up to 3 artefact double bonds in the aglycone structure. In order to achieve unaltered aglycons, several systems for enzymatic hydrolysis were tested. While β-glucosidase was not able to yield the macranthogenin aglycone, further experiments revealed pectinases and a glucuronidase to be powerful cleaving enzymes working under mild conditions (40 °C, pH 4.8). After precipitation, the aglycone fraction was re-dissolved and analysed via GC-MS and LC-MS. In addition to macranthogenin, two further diosgenin-like sapogenins namely sarsasapogenin/smilagenin and sceptrumgenin were detected. This study underlines that carefully chosen working conditions upon saponin hydrolysis are crucial to achieve an authentic aglycone profile in the course of phytochemical characterization of plant extracts.

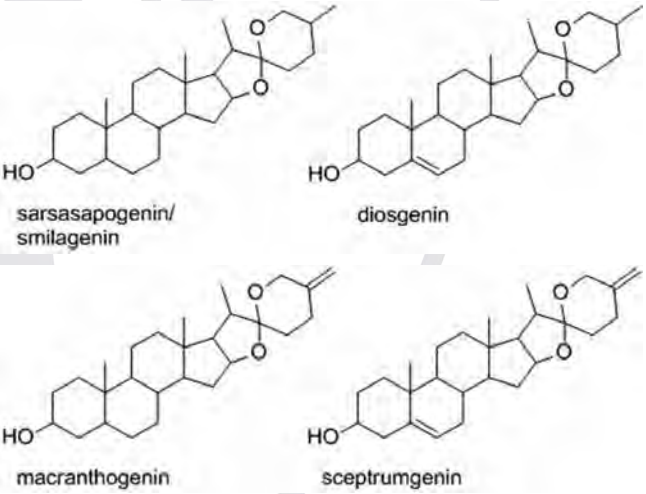

Fig. 1

References: [1] Man S., Gao W., Zhang Y., Huang L., Changxiao L. (2010) Fitoterapia 81: 703 – 714. [2] Linde, H.F., Isaac O., Linde H.H., Zivanov D. (1971) Helv Chim Acta 54: 1703 – 1708.

PK17 Qualitative and quantitative differentiation of species from *Vaccinium* by NMR spectroscopy in automation

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The genus *Vaccinium* contains approximately 450 species including bilberry, blueberry, cranberry, lingonberry, and huckleberry. The fruit of *Vaccinium* are widely eaten and touted for their health benefits, attributed to antioxidants and micronutrients. Blueberry leaf extract is a traditional medicine of the Cree people, believed to have anti-diabetic properties. Extracts from *Vaccinium* plants are commonly added to foods, dietary supplements, and cosmetics. Given their widespread use in humans, it is important to have methods to verify the identity and purity of *Vaccinium* extracts. Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for characterizing mixtures such as plant extracts in full automation. Discrimination of different species, absolute identification & quantification of key compounds and verification if the determined parameters are in the allowed range is the basis for the quality control of a sample. The automation of this process minimizes failures in the analysis. A comprehensive report makes the results easy to interpret. An NMR spectrum, such as a $1D^1H$ spectrum, shows the superposition of the characteristic signals of all of the compounds in the mixture. When enough samples of a specific material are available, chemometric models can be build. Then new samples can be classified against the model to determine whether they represent the same material. The result is represented in different ways, e.g. as quantile plot. Identifying and quantitating of key compounds from a mixture spectrum can be based on spectral analysis or using PLS-1. Both results can be used to verify if the sample is in the allowed range. We will demonstrate the power of these techniques using examples from our ongoing study of *Vaccinium*.

PK19 A validated method for the quality control of *Andrographidis* herba

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Andrographis paniculata (Burm. f.) Nees, (Acanthaceae) is used in Asian traditional medicine for the treatment of upper respiratory infections, lower urinary tract infections, diarrhoea, as hepatoprotective agent [1]. Recently it has become popular in Europe for the treatment of common cold [2]. Previous quality control studies concern the alcoholic extracts of *A. paniculata*. However, decoctions are often used in bioassays, while WHO indicates the use of decoction for fever and diarrhoea [1]. Therefore, quality control of the decoctions was additionally carried out in order to evaluate their content. An HPLC-UV method was developed and optimised for the determination of the main diterpenes. The method was fast, simple, effective and permitted the characterisation of all extracts. A Soxhlet (MeOH 100%) was applied to ensure exhaustive extraction of the plant material. Decoctions were prepared according to WHO. An RPC 18 column was applied using a gradient eluent system with an isocratic plateau consisting of acetonitrile and acidified water (pH = 3.2, formic acid); diterpenes were expressed as andrographolide (**1**). Resolution was satisfactory in all six comercial samples tested, most importantly between 14-deoxyandrographolide (**2**) and 14-deoxy-11,12-didehydroandrographolide (**3**). The method was validated in terms of linearity, intermediate precision (different days at three different concentrations) and accuracy. All validation criteria were fulfilled. Decoctions presented significant qualitative differences from the methanol extracts: in some cases isoandrographolide (**4**) was also detected in large amounts. Results underline the need to characterise preparations in addition to the herbal drug in order to evaluate therapeutical efficacy based on the qualitative and quantitative profiles of the administered preparations.
References: [1] WHO monographs on selected medicinal plants Vol 2, Geneve, 2002. [2] Kligler B, et al, (2006) Explore, 2, 25 – 29.

PK20 Phytochemical analysis of *Clerodendron trichotomum* by UHPLC-ESI-MS

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Clerodendron trichotomum Thunb. (Verbenaceae), widely distributed in Korea, Japan and China, has been traditionally used for the treatment of neuralgia, arthritis, cough and abdominal lump. Although water extract form (decoction) of the dried leaf and stem of *C. trichotomum* has been commonly consumed, there was no phytochemical analysis for determining the major constituents in the water extract. Therefore we performed phytochemical study on the water extract of *C. trichotomum* using UHPLC-DAD-ESI-MS technique. Four flavonoids, acacetin (*m/z* 285), clerodendrin (*m/z* 623), acacetin 7-glucuronosyl-glucuronide (*m/z* 637) and acacetin glucuronide (*m/z* 461) were identified as major constituents of the water extract by the comparison of their spectroscopic data with those in literatures. In this study, an UHPLC-ESI-MS fingerprint of *Clerodendron trichotomum* decoction was reported for the first time and the qualitative information on the major constituents was established for further studies on the quality control of herbal medicines and functional foods containing this species.

PK21 Characterization of phenolic compounds and cardiac glycosides from red sea squill (*Urginea maritima* (L.) Baker) by RP-HPLC-DAD-MS^n

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Sea squill (*Urginea maritima* (L.) Baker) is native to the Mediterranean area and has been used for treating heart diseases for a long time. Today, it is still used in homeopathy, mainly due to the presence of cardiac glycosides. Profiles of the latter have been intensively studied in the 1990ies [1; 2]. Common sample preparation strategies comprise tedious clean-up steps or compound isolation by column chromatography. Up until now HPLC methods have been merely focused on cardiac glycosides [3], although a wide range of further substances have been demonstrated to be also present in sea squill [4; 5]. In the present work, fresh plant material of red sea squill (Sardinia, Italy) was frozen, minced in a blender and extracted with aqueous acetone (90:10, v/v). Solid particles were removed by centrifugation (4560 *g*) and the resulting extracts were directly analyzed without further sample treatment. Separation was carried out with an RP-HPLC system coupled with DAD and MS^n detection and allowed to distinguish more than 50 compounds. Most substances were assigned to phenolic acids, flavonoids and cardiac glycosides, while dihydroquercetin and its hexosides were detected as predominant compounds. This simplified sample preparation procedure helps to characterize complex secondary metabolite spectra of sea squill, e.g. in screening studies without alteration of the genuine compound profile. Differentiation between various provenances and varieties is thus possible for authentication and selection of plant material upon quality control for pharmaceutical preparations.
References: [1] Krenn L, Kopp B, Deim A, Robien W, Kubelka W, *Planta Med* **60,** (1994) 63 – 69. [2] Krenn L, Ferth R, Robien W, Kopp B, *Planta Med* **57,** (1991) 560 – 565. [3] Kopp B, Krenn L, Jurenitsch J, *DAZ* **130,** (1990) 2175 – 2180. [4] Fernandez M, Vega FA, Arrupe T, Renedo J, *Galenica Acta* **24,** (1971) 45 – 57. [5] Vega FA, Fernandez M, *Naturwissenschaften* **51,** (1964) 483 – 484.

PK22 HPLC quantitative analysis of scutellarein tetramethyl ether: the active component of *Chromolaena odorata* leaf extract

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Chromolaena odorata (L.) King and Robinson (Siam weed) is a medicinal herb used for stop-bleeding, anti-inflammation and wound healing in tropical countries for centuries. It contains various bioactive components. Among them, scutellarein tetramethyl ether has been reported as a bioactive component for blood coagulation. In our previous study, it was shown as a bioactive component for anti-inflammation. In this study, we developed the HPLC analytical method for quantitative determination of this compound in *C. odorata* leaf extract. The method was validated for its linearity, precision, accuracy, limit of detection (LOD)

and limit of quantitation (LOQ). The separation was carried out using a Hypersil BDS C_{18}-column eluted with methanol: 0.5% acetic acid (60:40) with a flow rate of 1 mL/min and detection at UV 268 nm. Scutellarein tetramethyl ether showed a linear relationship within the range of 12.5 – 500 µg/mL. The method was shown to be precise with RSD < 2%. The average recovery was almost 100%. The average content of scutellarein tetramethyl ether in the extract was 102.9 µg/mL. The proposed HPLC method was appropriate for the analysis of scutellarein tetramethyl ether in *C. odorata* extract and would be useful for standardization of this plant extract.

PK23 Qualitative and quantitative analysis of tumor-promoting and skin irritating diterpene esters in homeopathic mother tinctures by HPLC and MS

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Some plants from the family *Euphorbiaceae* were used in the past as traditional remedies. With ingenol melbutate there is now a highly active therapeutic for the topical field therapy of actinic keratosis [1]. The active ingredients are diterpene esters, which are known to be tumor-promoting and skin irritating since Hecker et al. discovered TPA (12-O-tetradecanoylphorbol-13-acetate) in the oil of *Croton tiglium* L. seeds [2]. There are two basic chemical structures such as the alcohols phorbol and ingenol. These two alcohols form a great variety of different esters with different activities. Available preparations of these esters are homeopathic mother tinctures. For standardization and the monographic description in pharmacopoeias it is necessary to establish an easy method to quantify these esters from herbal preparations, e.g. homeopathic mother tinctures. To avoid the difficult analysis of the different esters and to quantify all the toxic esters it's necessary to hydrolyse them and to determine the resulting alcohols by HPLC. The alkaline hydrolysis is not suitable for all structures because of a bad recovery [3]. We will discuss the different approaches for gentle methods to hydrolyze the esters. The enzymatic hydrolysis with different enzymes such as esterase from *Bacillus subtilis* and the S9 fraction from rat liver ended almost in complete hydrolysis. For the identification of the different diterpene esters their peaks where collected after HPLC separation and then analysed by mass spectrometry. **References:** [1] Lebwohl, M. et al., *N Engl J Med* **2012**, *366*: 1010 – 1019. [2] Hecker, E., Schmidt, R., *Prog Chem Org Nat Prod* **1974** *31*: 377 – 467. [3] Gläser, S., *Untersuchungen zu einem möglichen Gesundheits- und Krebsrisiko durch pflanzliche Arzneimittel sowie industriell genutze Rohstoffe aus Euphorbiaceen – Quantitative Bestimmung von irritierenden und tumorpromovierenden Diterpenestern und Evaluierung durch biochemische und biologische Tests* (Thesis, Universität Heidelberg) **1991**

PK24 Determination of heavy metals in freeze-dried fruit samples with the AAS method

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Plants grown in soil that may be contaminated with heavy metals are an important entry point for toxic substances into the food chain. Information on how crops respond to soil pollution by individual metals is very much needed. This study examined the accumulation of Cd, Pb and Ni in samples of blueberry fruit, elderberry and chokeberry. Heavy metal contents were determined using the AAS method. Collected and freeze-dried fruit samples were weighed to 0.1 g. Prepared samples were mineralized and measured for cadmium, nickel, lead and compared to the standard at a wavelength of 226.50 nm (Cd), 220.35 nm (Pb), 231.60 nm (Ni). The Ministry of Agriculture and the Ministry of Health of the Slovak Republic by Decree 608/3/2004 – 100 shows the maximum permissible amount of chemical elements contained within a consumable item according to its smallest recommended dilution. The maximum allowable amount for Cd is 0.5 $mg.kg^{-1}$. The maximum for the Pb is 3.0 $mg.kg^{-1}$. The limit for the Ni content in foodstuffs is set at the level of 0.5 $mg.kg^{-1}$. After determining these heavy metals in the fruit and lyophilized blueberry, elderberry and chokeberry using AAS, we found that none of these limits were exceeded. The contents of heavy metals were determined by the force detection device, which is for lead 0.01 $mg.l^{-1}$, cadmium 0.001 $mg.l^{-1}$ and nickel 0.005 $mg.l^{-1}$. **Key words:** Heavy metal, Lyophilization, Mineralization, AAS. **Acknowledgment:** The work was supported by the Agency of Ministry of Education, science, research and sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/2010 – 11).

PK25 Analysis of volatile compounds in phytomedicines using thermal desorption gas chromatography mass spectroscopy

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Volatile essential oils (EOs) from medicinal plants play an important role in the treatment of various diseases. EOs have been shown to exhibit anti-bacterial, antiviral, antimycotic, antitoxigenic, antiparasitic, and insecticidal properties [1]. Several commercial phytomedicines such as Olbas oil (www.olbas.co.uk) [2], Tiger balm (www.tigerbalm.com) and Mentholtum contain EOs from medicinal plants such as peppermint. Thermal desorption (TD) gas chromatography mass spectroscopy (GC-MS) is a very useful technique for the analysis of volatile compounds without using tedious pre-treatment with organic solvents. Here we have analysed and compared the composition of volatile compounds of Olbas oil, Tiger Balm, and Mentholtum using TD GC-MS. Volatiles from each product have been identified and quantified. Menthol and eucalyptol have been found to be present in all the products (Figure). The easy and sensitive TD GC-MS method is useful for the quality control of phytomedicines containing volatile compounds.

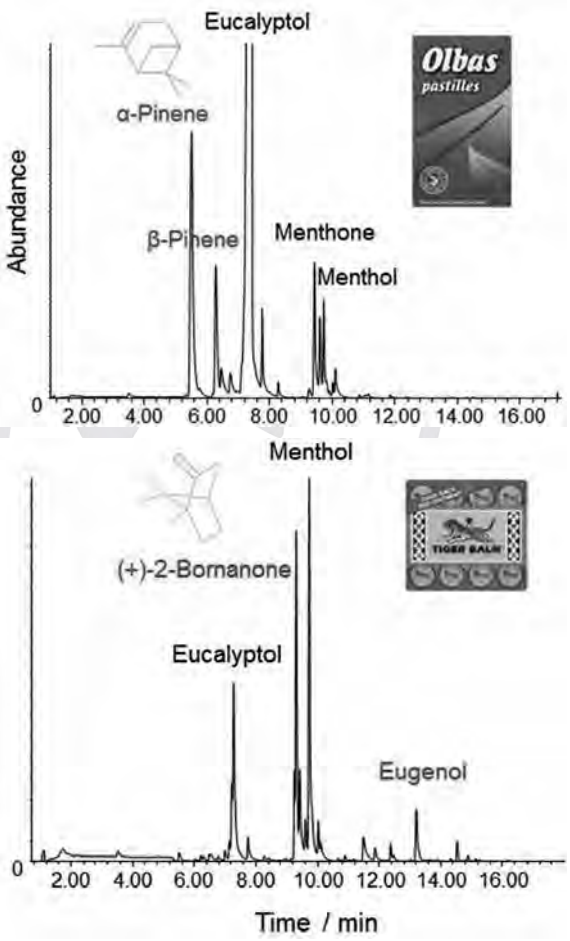

Fig. 1: TD GC-MS chromatograms

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PK26 Quality assessment microbiological parsley (*Petroselinum crispum*) and welsh onion (*Allium fistulosum*) leaves after drying process

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The parsley and Welsh onion are spices widely produced and consumed in Brazil. Their marketing is made of fresh or dried plant; where they are sell alone or together. This spices spices have medicinal properties, the parsley is considered diuretic and indicated for cardiovascular diseases and Welsh onion is antioxidant and for immune system. This study aimed to evaluate the drying of parsley and Welsh onion leaves at different temperatures (40, 50 and 60oC) and verify the microbiological quality of the plants before and after the drying process. The drying was realized in a dryer until the final water content of 0.11 in dry base. The microbiological quality of the leaves was performed for *Salmonella* sp.; mesophilics, molds/yeasts and *Escherichia coli*, according to Normative Instruction n° 62 of 2003 from Brazilian Ministry of Agriculture. The Brazilian microbiological food standard doesn't allow the presence of *Salmonella* sp. For *E. coli* is accepted until 10^2 CFU/g for fresh plant and 10^3 CFU/g for dried plant. The legislation does not provide limits for molds/yeasts and mesophilics, but some authors indicate 10^4 and 10^6, respectively. In this study, the count observed for yeasts/molds, mesophilics, *Salmonella* sp. and *E. coli* in fresh parsley were higher than those established by the Brazilian legislation and authors. It was observed that only on drying at 50 °C and 60 °C there was a reduction of the microbial population of the microorganisms analyzed.

PK27 Effect of seed pretreatment by H_2O_2 and magnetic field on the sensitivity of *Silybum marianum* L. to salinity sea water

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The effects of seed pretreatment by H_2O_2 and magnetic field (MF) on the impacts of sea water concentration were tested using *Silybum marianum* (*S. marianum*) at the vegetative stage. Some soaked *S. marianum* seeds were subjected to 0.18 T MF for various duration (0, 10, 20 and 30 min) and other seeds were soaked in different concentration of H_2O_2 (0, 80,160 and 240 μM) for 8 h. After germination the seeds were sowed in homogeneous soil and irrigated with tap water and 10% sea water concentration. Some effects of both MF and H_2O_2 pretreatment under sea water treatment were investigated. H_2O_2 pretreatment increased growth and development under control condition more than under sea water stress. On the other hand, our results provided evidence that seed H_2O_2 and MF-pretreatment increased the tolerance of *S. marianum* to salinity. In conclusion, our study demonstrated that sea water stress caused highly significant reduction in the growth parameters and stimulation in proline and phenolic compounds. Pretreatment seeds of *S. marianum* with H_2O_2 and magnetic field in different duration may alleviate the oxidative damage leading to improvements in physiological attributes for the plant growth under sea water stress. *Keywords: Silybum marianum*; Magnetic field; Physiological responses; H_2O_2, Sea water

PK28 Standardization and Bioassays Characterization on Malaysian herb, *Ficus deltoidea* Jack

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Standardization of herbal materials based on their chemical and biological profile is an important prerequisite for development of herbal product. Proper identification of the source plant with related biological property is the main issue encountered in herbal standardization. *Ficus deltoidea* Jack (FD) is a South East Asian native plant traditionally used to treat several diseases. Pharmacological data showed that this plant exhibited good antioxidant, anti-diabetic and anti-inflammatory properties. However, there are confusion on the identification of plant material as FD occur at least in eight varieties namely *Ficus deltoidea* var. *deltoidea,* var. *trengganuensis,* var. *kunstleri,* var. *montleyana,* var. *intermedia,* var. *bornensis,* var. *bilobata and var. angustifolia*. Since reports on pharmacological and chemical studies were not at the varietal level, it is difficult to identify the best variety to be commercialized. In order to determine the most biological active variety, eight varieties of FD were collected from several localities around Malaysian Peninsular and Borneo and subjected for HPTLC and HPLC fingerprinting analysis using biologically active marker compounds, vitexin and isovitexin. HPTLC chromatograms indicated significant differences in chemical composition among varieties with var. *deltoidea* and var. *trengganuensis* possess the most chemical content. Both varieties also contain the most phenolic compounds based on Ferric-chloride assay. The concentration of marker compounds also significantly different among varieties which showed that var. *trengganuensis* contains the highest concentration of both markers. *In vitro* antioxidant and anti-diabetic data revealed the differences in biological activities which var. *trengganuensis* and var. *motleyana* showed a comparable strongest activity compared to other varieties. Finding from this study will assist the selection of the best variety to be developed as herbal product.

PK29 Quantitative HPLC analysis of chamuangone in *Garcinia cowa* leaf extracts

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Garcinia cowa Roxb. ex DC. is a medicinal plant belonging to the family Clusiaceae. In Thailand, it is commonly known as "Chamuang". Our previous investigations reported an antibacterial activity of *G. cowa* leaf extracts against gastrointestinal pathogenic bacteria and cytotoxicity activity against *Leishmania major* and cancer cell lines. Purification of an ethyl acetate extract of *G. cowa* leaves using a bioassay-guided isolation afforded a new polyprenylated benzophenone, chamuangone (Figure 1), which exhibited satisfactory antibacterial activity against *Streptococcus pyogenes*, *Streptococcus viridans* and *Helicobacter pylori*, anyd *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus* sp. and had strong cytotoxic activities against *Leishmania major* and cancer cell lines, including A549, SCB3, K562, and K562/ADM. There was no quantification of chamuangone so far. In this study, chamuangone was used as an indicative marker for standardization of *G. cowa* leaf extracts using HPLC method. The method utilized a TSK-gel ODS-80Tm column (5 μm, 4.6 x 150 mm) with the mixture of acetonitrile and 2% v/v phosphoric acid in water (97:3, v/v) as the mobile phase at a flow rate of 1 mL/min, and UV detection at 245 nm. The parameters of linearity, intraday and interday precision, accuracy, specificity and sensitivity of the method were evaluated. The recoveries of the method were 100.4 – 101.6% and good linearity ($r^2 \geq 0.9999$) was obtained. A high degree of specificity, sensitivity as well as repeatability and reproducibility (RSD less than 2%) were also achieved. Microwave-assisted extraction (MAE) was selected as the best extraction method for chamuangone. The optimized MAE method was capable of increasing chamuangone content in the dried leaf extracts up to 11.9% w/w.

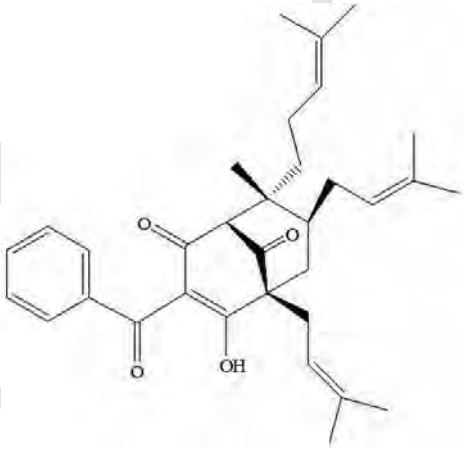

Fig. 1: Chemical structure of chamuangone.

PK30 Quantification by UPLC-MRM ESIMS of bufadienolides in *Bryophyllum pinnatum* leaves and manufactured products

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Bryophyllum pinnatum is a succulent perennial plant native to Madagascar. It is used in anthroposophical medicine to treat psychiatric disorders, and as a tocolytic agent to prevent premature labour. Besides flavonoids, the plant is known to contain bufadienolides, which reportedly exert sedative and positive inotropic as well as central nervous system-related activities. Despite the possible toxicological relevance of bufadienolides, no reliable data are available about their content in plants and phytotherapeutic preparations. In this context, a UPLC- ESIMS assay with multiple reaction monitoring (MRM) has been developed and validated for the quantification of the main four bufadienolides in *B. pinnatum*, bersaldegenin-1-acetate, bryophyllin A, bersaldegenin-3-acetate, and bersaldegenin-1,3,5-orthoacetate. The separation was performed on a Kinetex 1.7 uXB-C 18 column with a gradient of acetonitrile/water containing 10 mM ammonium formiate. Bufalin was used as an internal standard. Reference compounds were previously isolated from the related species *B. daigremontianum*. The method was used to determine the content of these four bufadienolides in *B. pinnatum* leaves of different geographical origins, and in manufactured products such as pressed juice and dried powder.

PK31 Development of an enzyme-linked immunosorbent assay for determination of iriflophenone 3-*C*-β-D-glucoside from *Aquilaria* spp. using a specific polyclonal antibody

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Aquilaria species (agarwood) are cultivated in Asian countries for the source of agarwood oil and endangered due to the scent industry. The leaves of *Aquilaria* spp. are used ethnomedically to treat anti-inflammatory conditions, diabetes, and as laxative [1 – 3]. The active compounds in *Aquilaria* spp. leaves are benzophenone glycosides including iriflophenone 3-*C*-β-D-glucoside (IP3G), iriflophenone 2-*O*-α-L-rhamnopyranoside and iriflophenone 3,5-*C*-β-D-diglucopyranoside [4]. Among them, IP3G is major active compound found in *Aquilaria* spp. leaves. Thus, a reliable and sensitive analysis method is necessary for quality control of the plant raw material and product. An enzyme-linked immunosorbent assay (ELISA) was developed using polyclonal antibody (PAb) against IP3G. The indirect competitive ELISA using anti-IP3G PAb was high specific for IP3G. There were low cross-reactivity with iriflophenone 3,5-*C*-β-D-diglucopyranoside (13%), genkwanin 5-*O*-β-primeveroside (3.55%) and no cross reactivity found in other compounds. The range of ELISA assay extends from 100 – 1,560 ng/ml with coefficient of variation (CV) 1.19 – 2.07% for intra-assay and 3.76 – 5.70% for inter-assay precision levels. The percentages of recovery in the range of 96.0 – 99.0% with CV of 4.50 – 5.32%, which indicated that the method is accurate in agarwood samples. The correlation between the result from the ELISA and HPLC had a coefficient of determination (r^2) of 0.9321. The developed immunoassays using the PAb against IP3G are simple, reliable, sensitive and cost-effective assay for quality control of agarwood raw material and product. **References:** [1] Zhou MH, Wang HG, Suolangjiba Kou JP, Yu BY. 2008. J Ethnopharmacol 117:345 – 50. [2] Kakino M, Izuta H, Ito T, Tsuruma K, Araki Y, Shimazawa M, Oyama M, Iimuna M, Hara H. 2010. Biosci Biotechnol Biochem 74:1550 – 5. [3] Pranakhon R, Pannangpetch P, Aromdee C. 2011. Songklanakarin J Sci Technol 33:405 – 10. [4] Feng J, Yang XW, Wang RF. 2011. Phytochemistry 72:242 – 7.

PK32 Evaluation of food composition and safety in nutraceutical products from green tea (*Camellia sinensis*) by uhplc-orbitrap-ms and gc-qqq-ms/ms

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Nutraceuticals consumption has increased in the global market. One of the problems related to their development is the appearance of fraudulent products. Therefore, the Food and Drug Administration (FDA) approved in 1994 the law Dietary Supplement Health and Education Act (DSHEA). Another issue relies on the fact that nutraceuticals are produced from herbal concentrates, so the occurrence of pesticides used during herbal production is possible. In the European Union (EU), Regulation EC 396/2005[1] control pesticide residue levels, but only in raw material. Many nutraceuticals are derived from tea, mainly green tea, which has a major amount of polyphenols. However, the information shown in the label is not complete and nutraceutical composition is not well specified. Obviously, polyphenol composition will determine healthy effects derived from their consumption. In this study, polyphenols and pesticides were determined in nutraceuticals derived from green tea in order to assure the quality and safety of this type of products. For that purpose, chromatrographic techniques (gas and liquid chromatography) coupled to mass spectrometry analyzers (triple quadrupole and Exactive-Orbitrap) were used. Regarding polyphenols content, higher concentrations were observed for aglycones (-)-epicatechin, (+)-catechin, gallic acid, (-)-gallocatechin, and quercetin-3-*O*-rutinoside, in the range from 261 to 10729 mg kg^{-1}, which are similar to previous studies. Concerning nutraceutical safety, more than 150 pesticides were monitored. Pesticides as 4,4-dichlorobenzophenone, chlorpyrifos methyl, o,p'-dicofol, p,p'-dicofol and phthalimide, were detected in the samples at concentrations ranging from 27.5 to 44.5 μg kg^{-1}. **Acknowledgements:** The authors gratefully acknowledge the Spanish Ministry of Economy and Competitiveness and FEDER (Ref. CTQ2012 – 34304). GMD acknowledges the Health Secretary from Veracruz, Mexico and the Mexican Senate for financial support. **References:** [1] REGULATION (EC) Nº 396/2005

PK33 New HPLC – method to determine Frangulin A and B as well as Glucofrangulin A and B in Frangulae cortex

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Introduction: The current monograph in the European Pharmacopeia for Frangulae cortex describes a photometric assay based on an adapted bornträger reaction to determine hydroxyanthracene glycosides, calculated as frangulin B. The method is time consuming, unspecific for frangulines and the precision is not adequate for a modern assay. **Aim:** The photometric method shall therefore be replaced by a modern HPLC-method. There is no HPLC method published in the literature that allows the determination of frangulin A/B and glucofrangulin A/B in *Frangulae cortex*. **Method:** About 300 mg of freshly milled drug are extracted for 15 min with ultrasound. The extraction solution consists of acetonitrile/water 50:50 v/v and 2 g/L $NaHCO_3$. A conventional RP C_{18} Nucleodur (4 mm x 125 mm), 3 μm was used as stationary phase. Mobile phase A consists of water (pH of 2.0, adjusted with phosphoric acid). Mobile phase B consists acetonitrile/methanol 20:80 v/v. The flow rate is 1.0 mL/min, the detection wavelength 435 nm, the column temperature is 50 °C, and the injection volume 20 μL. The gradient is shown in table 1.

Tab. 1: Gradient table

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	66	34
15.0	66	34
16.0	50	50
26.0	24	76
26.5	0	100
28.5	0	100
29.0	66	34
45.0	66	34

Results: The mobile phase separates the four frangulins sufficiently. Results of several samples will be presented on the poster. A chromatogram from a Frangulae cortex sample is shown in figure 1.

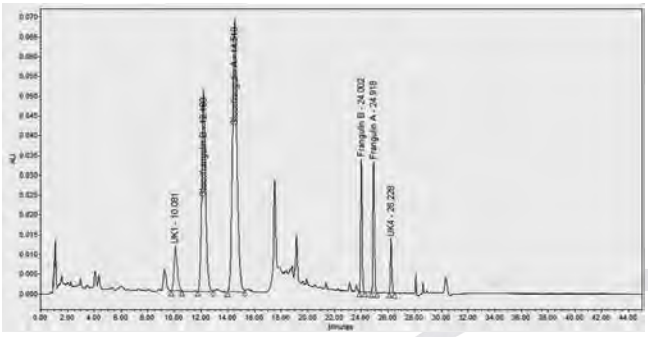

Fig. 1: Chromatogram from a Frangulae cortex sample.

Conclusion: The method we developed is simple, robust and precise. It is a reasonable option for pharmacopeia applications to replace the outdated photometric assay.

PK34 New UHPLC – method to determine Aloin A and B in *Aloe capensis*

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Introduction: The current monograph in the European Pharmacopeia for Aloe capensis describes a photometric assay based on an adapted bornträger reaction to determine hydroxyanthracene glycosides, calculated as aloin A. The method is time consuming, unspecific for aloin A and B and the precision is not adequate for a modern assay. There are several HPLC methods published, but their runtime is too long and the resolution for aloin A and B is not satisfactory. There is no validated and robustness checked method at all. **Aim:** The aim of the present study was to develop a short, robust and validated UHPLC method that meets specific needs of the pharmaceutical industry, this means short retention times and high repeatability. The method is suitable for the analysis of herbal drug and herbal drug preparation. **Method:** About 100 mg of the drug are placed in a 100 mL volumetric flask and extracted with 70 mL of methanol for 20 min with ultrasound. An Acquity UHPLC BEH Phenyl Column (2.1 x 50) mm, 1.7 µm was used as stationary phase. The mobile phase consists of 17% acetonitril, and 83% water. The flow rate is 0.5 mL/min, the detection wavelength 355 nm, and the injection volume 3 µL. The method was validated according to ICH guidelines. **Results:** The mobile phase separates aloin A and B. Results of several samples will be presented on the poster. A chromatogram from a *Aloe capensis* sample is shown in figure 1.

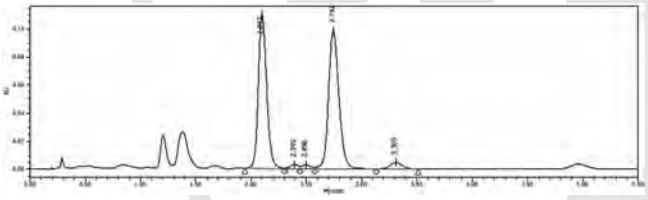

Fig. 1: Chromatogram of a *Aloe capensis* sample

Conclusion: The method we developed is simple, robust and precise. The method is also available for normal HPLC systems. It is a suitable option to replace the outdated photometric assay described in the European Pharmacopeia. **Keywords**: UHPLC, *Aloe capensis*, aloin A, aloin B

PK35 Ethnobotanical, physiological, histological study and evaluation of the contents of Omega-3 of *Portulaca oleracea* of the area of Djelfa (Algeria)

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The objective of this study is the knowledge of the Algerian natural resources in producing plants of fatty-acids "Omega-3" and the evaluation of their capacity to produce for their possible industrial exploitation, while being interested in the extracts of a species of the family of Portulacaceae: *Portulaca oleracea*. In our study a certain number of objectives were set: a ethnobotanic study of *Portulaca oleracea* in order to better know and to develop the uses of this species; A histological study of the various bodies; A chemical screening and a test of germination to know the best conditions of the germination of seeds. We determined and analyzed the contents of Omega-3 by Mass Spectrometry coupled to a Chromatograph in Gas Phase (CPG/SM-QP 2010). The results show a variability of the contents of fatty-acids (Omega-3) between the bodies collected at the various periods of the year, whose content of the Omega 3 of the bodies collected in July is twice more important than those collected in May. Diallo and *al.*, (2010), cites several factors that influence the composition of fatty acids, like the light and the type of soil. *Portulaca oleracea* is rich in active principles such as: alkaloids, flavonoids. The germination of seeds of *Portulaca oleracea* is highly significantly affected by the temperature and significantly by the size of seeds. The histological study showed that the organs of *Portulaca oleracea* are rich in calcium oxalate and potassium crystals. Schauenbergand Paris (1977), reported the presence of crystals of calcium oxalate and potassium oxalate in the organs of *Portulaca oleracea*. **References:** [1] Diallo F. B., Begin D., Gerin M., 2010. Knowledge reviews: The solvent substitution by methyl esters of vegetable oil fatty acids. IRSST: B-079. [2] Schauenberg P. and Paris F., 1977. Le piante medicinali, Roma, Newton Compton.

PK36 Identification of selected Thai medicinal plants by PCR-RFLP method

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Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to generate DNA marker for identification of the selected medicinal plants that closely resemble in morphological and histological characters. In the PCR-RFLP method, fragments of DNA are amplified from each sample with a set of specific primers and they are further examined for their nucleotide sequences to provide information for restriction enzyme cut site. Finally, DNA fragmentation pattern is generated by selected restriction enzymes. *Murraya koenigii*, *Micromelum minutum* and *Clausena excavata* were classified in family Rutaceae. *Murraya koenigii* which is reported to possess antioxidant, anticarcinogenic, anti-lipid peroxidetive, hypoglycemic and hypolipidemic properties [1] can be distinguished from *M. minutum* and *C. excavata* by the presence of 2 fragments of DNA after amplification of the internal transcribed spacer (ITS) of nuclear ribosomal RNA gene (rDNA) followed by digestion with restriction enzyme *Bsr*BI. Amplification the ITS sequence and fragmentation with the restriction enzyme *Apa*I results in fingerprint pattern that is useful in discrimination of *Zanthoxylum myriacanthum* (Rutaceae) and *Zanthoxylum limonella*. *Z. limonella* and not *Z. myriacanthum* is reported to exhibit antimalarial and antituberculous activities [2]. PCR-RFLP based on maturase K gene sequence and *Pvu*I digestion was proved to discriminate *Kaempferia parviflora* (Zingiberaceae) from *Kaempferia galanga* and *Kaempferia marginata*. *K. parviflora* has been reported to possess cytotoxicity against human cholangiocarcinoma cell lines [3]. **References:** [1] Tachibana, Y. et al. (2001) J. Agric. Food Chem 49:5589 – 5594 [2] Charoenying, P. et al. (2008) KMITL Sci. J. 8:12 – 15 [3] Leardkamolkarn, V. et al. (2009) Asian Pac. J. Cancer Prev.10:695 – 8.

PK37 The quality of commonly used food supplements on the Belgian Market.

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In Belgium, food supplements are regulated by the Royal Decree of August, 29th, 1997 which was modified April, 4th, 2012 [1,2]. This Decree describes the notification procedure that has to be completed before a market authorization can be obtained next to other criteria that have to be fulfilled such as labeling and advertisements. The third annex of this document is a list of plants allowed to be commercialized as food supplements. For some of these plants maximal daily doses were established by the Advisory Committee for Plant Preparations. In table 1 these limits are shown for *Humulus lupulus*, *Hypericum perforatum*, *Ginkgo biloba* and *Glycine max*. Each batch of herbal preparations based on these plants should be checked for their compliance to this guideline. The list also proposes suitable methods for these analyses, which should be proven to be applicable to the preparation by the manufacturer. Over

the past five years, more than 320 samples of these four herbal preparations were analysed. Only 35% was found to be compliant. There are four main reasons for non-conformity: 12% exceeds the maximal allowed daily dose; 34% did not comply with the limits of content. For over 15% of the samples the content was not specified, consequently compliance of the content could not be determined. Only 1% was found to be adulterated. Additionally we encountered many problems in applying the standard methods to the complex preparations and specific matrices. In conclusion, there is an urgent need for quality control of food supplements by the manufacturer.

Plant	Marker	Maximal daily dose	Number of samples	Number of samples exceeding maximal daily dose	Number of samples non-compliance of content
H. lupulus	8-pregnyl naringenin	400 µg	75	0	16
H. perforatum	Hypericin	700 µg	67	12	32
G. biloba	Flavonolglycosides Terpene lactones	21.6 mg 5.4 mg	96	5	35
G. max	Isoflavones	40 mg	82	20	25

References: [1] https://portal.health.fgov.be/portal/page?pageid=6,513211&dad=portal&schema=PORTAL. [2] http://www.health.belgium.be/eportal/foodsafety/foodstuffs/foodsupplements/index.htm#Planten

PK38 Surveilance of possible adulterants in herbal sliming products in bosnian market

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On the world markets there is large and still growing number of herbal sliming products, that promise fast and easy loss of weight. Such products are easily available to consumers through different distribution channels: pharmacies, supermarkets, via internet etc. They are declared and registered as dietary products, and as such, they don't undergo rigorous testing and control intended for medicines. Due to this, once in a while a product reaches market with added undeclared substances. In this study, we tested 9 herbal sliming products available in pharmacies in Bosnia and Herzegovina on presence of undeclared substances: theophyline, ephedrine, metformin, caffeine, phenolphthalein and sibutramine (Table 1.)

Tab. 1: Overview of analyzed samples

Sample N°	Country of origin	Dosage form	Labeled API(s)
01	Serbia	powder for oral suspension	chitosan, chromium, lyophilized pineapple
02	Serbia	herbal mixture for tea preparation	*These folium, Millefolii herba, Frangulae cortex, Sennae folium, Foeniculi fructus, Menthae piperitae folium, Taraxaci radix, Betulae folium, Juniperi fructus, Melissae folium, Cyani flos*
03	Italy	tablets	dry extract *Ananas comosus* (L.)
04	Serbia	tablets	Clove essential oil, Cinnamon essential oil, Ginger essential oil
05	China	capsules	extracts: Folium Nelumbinis, Rhizoma Dioscoreae, Poria, Fructus Crataegi
06	Croatia	capsules	Natural brown seaweed extract, extract from red orange, regular orange, grapefruit and guarana
07	Denmark	tablets	Chili Pepper, Green Tea, Dill seed, Ginger. Peppermint oil
08	Austria	capsules	Pineapple extract, Asparagus extract
09	Slovenia	powder for oral suspension	chlogenic acid, chromium

Validated UPLC method was used with gradient elution and mobile phase consisting of mixture of acetate buffer and acetonitrile (1.). Samples were prepared by extracting amount of product equivalent to one dose with 25 ml 20% acetonitrile. Identification was performed by comparing retention times of UV spectra of suspicious peaks with those of standards. The tested undeclared substances were not found in the tested products. Even so, there is an increased interest in this type of products by government institutions after scandal with added sibutramine in one sliming product a few years ago (2.). The need for constant monitoring of food supplements must be stressed here, especially in the markets with "gray areas" in existence. **Reference:** [1] E. Deconinck, K. Verlinde, P. Courselle, J.O. De Beer, A validated Ultra High Pressure Liquid Chromatographic method for the characterization of confiscated illegal slimming products containing anorexics. J Pharm Biomed Anal 2012; 59: 38 – 43. [2] Napitak zelena kafa povučena sa tržišta u Srbiji http://www.blic.rs/Vesti/Drustvo/179525/Napitak-zelena-kafa-povucena-satrzista-u-Srbiji> (Accesed March 2, 2013.)

PK39 Chromatographic examination of *Aremonia agrimonoides* (L.) DC (Rosaceae)

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Aremonia agrimonoides,(L.) DC (Rosaceae), is a native plant species in central Europe. As the continuation of previously performed research [1], this work represents a research by chromatographic dry herbal material. Plant materials were collected in Bosnia and Herzegovina during May 2011. Two HPTLC methods were performed. The first method was described by Harborne [2] and the second method was described by Lee and others [3]. The third method was two dimensional TLC on Cellulose MN300 plates as described by Harborn [2]. Spot detection was observed under UV light and by spraying with: vanillin/HCl reagent, sodium nitrite reagent, saturated KIO_3 water solution, Folin-Ciocalteu reagent. In table 1. we present our results.

Tab. 1: HPTLC spots with vanillin/HCl reagent (red spots) and Folin-Ciocalteu reagent (grey spots)

Method	Part of herb	Rf (catechin standard Sigma Aldrich)	Rf	(Rf (procyandidin B1 Sigma Aldrich)	Rf	Rf
HPTLC method 1	Aremoniae herba	0.81	–	0.63	–	–
	Aremoniae rhizoma cum radicibus	0.81	–	0.63	0.43	0.35
HPTLC method 2	Aremoniae herba	0.45	–	0.27	0.18	–
	Aremoniae rhizoma cum radicibus	0.45	0.32	0.27	0.18	0.11

In TLC method with Cellulose MN300 plates after spraying with saturated KIO_3 we observed a spot with brown color on start line (possible ellagitannins). In table 1. we present spots of catechins (react with Folin-Ciocalteu (phenolics) and vanillin/HCl reagent (catechins). In rhizome and herb we find catechin, dimers and trimers of catechin. on Rf 0.32 according to Lea it is possible to be procyanidin B5. *Aremonia agrimonoides*, (L.) DC (Rosaceae) possesses anti-inflammatory activity (1) and can be possible source of catechins. **References:** [1] Pilipovic S, Mulabegovic N, Mornjakovic Z, Uzunovic A, Elezovic A, Hadzidedic S: Anti-inflammatory activity of ointments with dry extracts of rhizome and herb of Aremonia agrimonoides, (L.) DC (Rosaceae). Planta Med 2011; 77: 1430 [2] Harborne JB. Phenolic compounds In: Phytochemical methods: A guide to modern techniques of plant analysis. 3 rd ed. Harborne JB. London, Chapman & Hall. 1998; 40 – 106. [3] Lea AGH, Arnold GM. Phenolics of ciders – bitterness and astringency. J. Sci. Food Agric. 1978; 29: 478 – 483

PK40 Evaluation of dissolution properties of silymarin capsules at different pH values

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Milk thistle (*Silybum marianum* L.) is a medicinal plant widely used in traditional medicine. Silibinin is the major and most active component present in silymarin, a polyphenolic flavonoid isolated from milk thistle. The aim of the present work is to compare the influence of surfactant or acidity varying on dissolution profiles of silymarin capsules, obtained from one producer, label claim 100 mg of silibinin. Silibinin working standard was provided by Sigma-Aldrich (Buchs, Switzerland). Differences in the dissolution properties of silymarin capsules were determined in four dissolution media: 0.5% sodium lauryl sulphate (SDS) in water, simulated gastric fluid pH 1.2, phosphate buffer pH 4.5 and phosphate buffer pH 6.8. Fixed volumes of the dissolution medium were withdrawn at 15, 30, 45 and 60 minutes. Dissolution tests were performed on the USP Apparatus 2 (Dissolution tester ERWEKA DT 800; rotating speed 100 rpm at 37 ± 0.5^0C, 900 mL) [1]. HPLC method proposed by Wu et al. was used for the determination of the amount of the active ingredient released [2]. HPLC was performed with a mobile phase composed of 0.01 M monosodium phosphate buffer pH 5.45 ± 0.05:acet-

onitrile ($50:50_{v/v}$), and peaks were detected at 288 nm. Degassed and diluted samples were analysed on LiChrospher® C18 column (250 x 4.0 mm, 5 µm), at 24 ± 1 ^{0}C and 1.0 mLmin^{-1} flow rate. The dissolved amounts of silibinin at the end of testing were in the range of 1.4 ± 0.2% at pH 1.2 to 40.1 ± 5.3% at 0.5% SDS. The results of dissolution studies are summarized in Figure 1. On the basis of our results, it can be concluded that presence of surfactant or physiological variation of pH of the gastrointestinal tract could influence dissolution rate of silibinin from silymarin capsules.

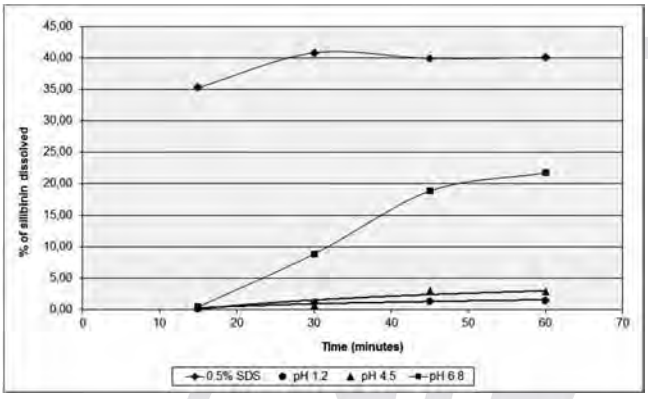

Fig. 1: Comparative display of dissolution profile for silymarin capsules

References: [1] Campodonico, A. et al. (2001) Drug Dev Ind Pharm 27(3): 261 – 265. [2] Wu, J.-W. et al. (2007)J Pharm Biomed Anal 45:635 – 641.

PK41 Optimisation of *Echinacea purpurea* extraction and processing to yield high potency antiviral activity

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Introduction Antiviral and anti-inflammatory activities are important for the prevention and treatment of respiratory tract infections. These activities can be found in the herbal medicinal plant *Echinacea purpurea* (EP). However different commercial preparations of EP vary greatly in chemical composition and the manufacturing procedures used, and consequently differ significantly in their antiviral potencies. As a result, there is no standard procedure for the preparation of consistent high potency extracts. In this study we evaluated different types of EP extracts for relative antiviral activities. **Antiviral activity** Ethanol is an ideal solvent to isolate the antiviral principles from *E. purpurea* (1). We separately investigated ethanol extracts (65% V/V) of roots, herb (aerial part without flowers), flower heads and the petals. Minimal inhibitory concentrations (MIC) were measured quantitatively by means of standard plaque assays with influenza virus type A, H3N2 (1). No antiviral activity was found in the roots and the petals (MIC > 1 mg/ml); but extracts of the herb and of the flower heads were active (MIC < 26.5 µg/ml). In addition there was a substantial difference between the freshly processed herb providing tinctures with MIC < 2.3 µg/ml in comparison to dried herb tincture with MIC equal to 16.8 µg/ml. **Anti-inflammatory activity** Previous studies have demonstrated the anti-inflammatory potential of alkylamides that are enriched in roots of Echinacea species. Thus a combination of herb and root tinctures, prepared from freshly harvested plants, would provide the full spectrum of pharmacological activities for successful cold management. **Conclusion** Ethanol extracts from *E. purpurea* herb and roots have distinct antiviral and anti-inflammatory potential. In order to obtain optimal benefits of EP, it is desirable to use both herb and root components, derived from freshly harvested plants. **References:** [1] Vimalanathan S, et al. (2005). *Pharm. Biol* 12;43(9):740 – 745.

PK42 Characterization and specification finding of *Mucor racemosus* preparation used as homeopathic starting material

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Homeopathic medicinal products produced from preparations of *Mucor racemosus* Fresen. (DSM 2845) have been used for over 30 years (Mucokehl®, Vetokehl Muc ®). For the production of the *Mucor racemosus* starting material, the biomass yielded by fermentation is purified and then mechanically opened by a cell mill. The liquid phase is separated and the insoluble components are filtered. The filtrate then undergoes multiple filtration and washing steps prior to sterile filtration and freeze drying. The resulting starting material is named "e volumine cellulae (lyophil., steril.)" evc [1]. For many years a Capillary Electrophoresis (CE) method is used for identity characterization of the homeopathic starting material [2]. Due to the variability of the biological material and even though the process is strictly GMP controlled to have a batch to batch consistency, peaks differ in their height. Sometimes small peaks disappear. It is obvious that used CE method alone is to detailed to indicate identity of Mucor racemosus evc for GMP purposes. Also a very exact rational has to be defined for assessment during batch release. In lack of this a more robust method for specification is demanded. A SDS-PAGE method has been developed and validated with the homeopathic starting material Mucor racemosus evc. Calculations are shown to constitute a specification via SDS-PAGE. Relative standard deviation is tabulated and demonstrates an excellent value in respect to this biological material. Different analytical methods (e.g. protein content, carbohydrate composition of polysaccharides) are presented to characterize the homeopathic starting material Mucor racemosus evc. Due to the GMP- controlled process of many years and the presented analytical data a good justification of specification can be concluded. **References:** [1] Bader, G., Akkoyun, A., Wiethoff, K. (2010) Planta Med. 76:1262 [2] Wiethoff, K., Irmer, A., Bader, G., (2010) Planta Med. 76:1331

PK43 PCR-based authentication of commercial black cohosh products – implications for reported hepatotoxicity

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Black cohosh (BC) (*Actaea racemosa*), is a popular herbal remedy to treat side effects of the menopause. In 2011 US sales were over $10,000,000 and it ranked at number eight amongst the most popular herbs. However, its credibility is being damaged by links to reported cases of hepatotoxicity, although investigation of these reports has not been able to confirm that BC plant material is responsible. This has raised the suspicion that some cases of adverse reactions may result from substitution or adulteration with Asian *Actaea* species, rather than to *A. racemosa*. This demonstrates the need for correct identification of *A. racemosa* in BC products, and the exploration into potential hepatotoxicity of these different *Actaea* species. We report the development of specific PCR-based assays capable of discriminating *A. racemosa* from potential adulterant species, particularly those associated with hepatotoxicity. A group of closely related *Actaea* species were chosen based on the knowledge of use as an adulterant in BC preparations. Species-specific primers were developed by aligning the DNA sequences and selecting areas of variation unique to each species. The primers were optimised for both multiplex PCR and qPCR. The product from each reaction was designed to differ in size to enable their resolution by capillary electrophoresis in the multiplex assay, and they were shown to amplify with high efficiency in qPCR assays. These assays were used to authenticate several commercially available products. DNA was isolated from a range of different products and specific *A. racemosa* DNA sequences could be detected in most of the products tested. Tests are now underway to detect adulterant *Actaea* species in the same products. Hepatotoxicity assays using cultured cells are being developed to test preparations from a number of *Actaea* species. These results will indicate the relative hepatotoxicity of BC itself and of adulterant *Actaea* species detected by DNA authentication assays.

PK44 HPTLC method for phytochemical and radical scavenging profiles of Chilean "Maqui" berries (*Aristotelia chilensis*) at different ripening stages from cultivated accessions

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Aristotelia chilensis (Molina) Stuntz is a shrub which grows wild in central to southern Chile and western Argentina. Apart from its traditional medicinal use in Chilean folk medicine, it is also known for producing tasty antioxidant berries, locally called "Maqui". The exploitation of the wild resources has grown extensively. Selection, domestication and cultivation studies on an agroindustrial scale are ongoing in Chile in order to provide industry with sustainably produced Maqui berries with standardized quality characteristics. HPTLC was chosen for the assessment of phytochemical and antioxidant variations in Maqui berry samples of different genotypes, field cultivation conditions and ripening stages at harvest time. The sample preparation method described in [1] was modified for small sample amounts using mechanically assisted extraction with ceramic beads. A suitable HPTLC method was developed using derivatization with Fast Blue Salt and Natural Product reagents for phytochemicals and DPPH for radical scavenging activity profiles. In addition, a high throughput ORAC assay was used for comparison of antioxidant activity in the samples. The results suggest that differences in ripening stages and cultivation conditions lead to variations in phytochemical and radical scavenging profiles on HPTLC plates supported by antioxidant ORAC data. The study is an example of how HPTLC can be a powerful, rapid and cost-effective tool in both quality control and the support of agronomic research on valuable medicinal and neutraceutical plants. Acknowledgments: FONDEF Chile (Cultivation project) and KFH-DC Switzerland (MaquiSelect). **References:** [1] Escribano-Bailón M, Alcalde-Eon C, Muñoz O, Rivas-Gonzalo J, Santos-Buelga C (2006): Phytochemical Analysis 17:8 – 14.

PK45 Early Growth and Physiological Characteristics of *Parasenecio firmus* by Shading Conditions in Forest Farming

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Parasenecio firmus is a perennial plant in *Asteraceae*, *Parasenecio* that distributed in Korea, China, and Japan. As dietary style changes for well-being life, consumer demand for functional wild vegetable is increasing. *P. firmus* has great fragrance and texture and contains caffeoylquinic acid. It has the potential to be further developed as a functional wild vegetable. However, as the natural habitat of *P. firmus* is quite limited and it should be collected manually in natural stand. So it is need to be a scientific research for artificial cultivation in forest. This study was conducted to investigate the optimum light conditions of *P. firmus* in forest farming. In this experiment, *P. firmus* was grown under four different light conditions (50%, 30%, 20%, and 10% of full sunlight) and non-treated (full sunlight). Out of five different shading conditions, *P. firmus* has shown the best physiological and growth responses to 10% and 20% of full sunlight. There is no statistically significant difference in other shading conditions. At 10% of full sunlight, *P. firmus* has shown the highest level of statistical significance in the length of shoot, fresh weight of shoot, water contents in all part, SLA (specific leaf area) and LAR (leaf area ratio). It indicates higher water content level, thinner leaf, and wider light-interception areas. On the other hand, at 20% of full sunlight, root color diameter, root length, total fresh weight, total dry weight, leaf area, and chlorophyll contents turned out to be high. It shows that *P. firmus* is active in chlorophyll activities and carbon dioxide assimilation at even lower light conditions. These results suggest that the optimum light level of *P. firmus* for artificial cultivation ranges from 10 to 20% of full sunlight. These results can be used as basic data for forest farming.

PK46 Comparative analysis of polyphenols and caffeine contents of espresso coffee extracted with differently purified water

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Coffee is one of the most popular beverages in the world. The flavor and taste of coffee are known to be varied by different cultivation region, the contents of bioactive compounds such as polyphenols and caffeine also varied according to the cultivation environment. As the extracting solvent for the espresso is water, and there are various types of water purification methods, we tried to see if there were any differences the chemical profile of coffee by the extracting water-type in terms of the contents of major components. In the present study, we extracted roasted coffee with differently filtered water (hollow fiber membrane, reverse osmosis pressure and electrolytic method). Ten major polyphenols such as 3- caffeoylquinic acid (CQA), 4-CQA, 5-CQA, 3- feruloylquinic acid (FQA),4-FQA,5-FQA, etc. and caffeine in the extracts were analyzed by ultra high performance liquid chromatography with photodiode array detector. The polyphenols and caffeine were identified by the comparison of their chromatographic and spectroscopic data with those in literatures. Relative contents of total polyphenols and caffeine were calculated by the sum of integrated peaks in the chromatogram. The pHs of filtered water were recorded before the extraction and all the data obtained in the present study were analyzed using the Student's t test to determine whether the differences were statistically significant. The result showed that electrolytic method had more extraction efficiency for the total polyphenols and caffeine than those of other methods. These results indicated the flavor and taste of coffee also be affected by the type of water filtration used for coffee machine.

PK47 Validation of UPLC method for determination of rubrofusarin-6-O-gentiobioside from *Cassia obtusifolia* L.

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The seed of *Cassia obtusifolia* L. contains various kinds of anthraquinone, anthraquinone glycosides and naphthalene derivatives and is widely used in Asia to treat eye inflammation. Also, the extract of *Cassia obtusifolia* L. was known to reduce the memory impairments and neuronal damage caused by 2VO in a mouse transient global ischemia model. Rubrofusarin-6-O-gentiobioside, one of a major component of *Cassia obtusifolia* L., has been known to be an important constituents of this herbal drug with a lot of biological acitivities including decrease of the expression of TGF-β1 and fibronectin and NF-κB DNA binding activity and radical scavenging effect. However, there was little analytical method for determination of rubrofusarin-6-O-gentiobioside in *Cassia obtusifolia*. In this study, a rapid and simple UPLC method with UV detection was developed and validated to determine rubrofusarin-6-O-gentiobioside in *Cassia obtusifolia*. The method was established on a reversed phase C 18, Brownlee column (2.1 mm x 75 mm, 2.7μm) and detection wavelength was 281 nm. The mobile phase was acetonitrile (0.1% trifluoroacetic acid) and water (0.1% trifluoroacetic acid) with an isocratic elution and sample analysis time was 8.0 min. UPLC method is validated in terms of linearity range, limit of quantification, selectivity, accuracy and precision. The retention time of rubrofusarin-6-O-gentiobioside was 2.1 min. Calibration curve showed good linearity (R^2= 0.999) over the range of 5.0 – 500.0 μg/mL. The relative standard deviation for intra- and inter-day precision was found to be below 3%. The recovery rate ranged from 105.0% to 114.0% and limit of quantification was 7.0 μg/mL. This method can be applied to determine rubrofusarin-6-O-gentiobioside rapidly and be utilized to control the quality of *Cassia obtusifolia* L..

PK48 Enzyme-linked immunosorbent assays for quality control of miroestrol and deoxymiroestrol in *Pueraria candollei*

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Miroestrol (ME) and deoxymiroestrol (DME) are the most potent estrogenic compounds in *Pueraria candollei*, which has been applied for long time in Thai folk medicine. The clinical trials showed that menopausal symptoms were declined significantly after treatment with different doses of *P. candollei* var. *mirifica*, whereas no significant side effects were observed. Therefore, standardized plant materials of ME and DME are necessary to researches and industrial production involved with *P. candollei*. Indirect competitive enzyme-linked immunosorbant assays (ELISAs) for determination of ME and DME were developed and validated by using polyclonal antibodies (PAbs) from rabbits. The PAb against ME recognized specifically to ME, which exhibited cross-reactivity to DME and isomiroestrol with 6.68% and 1.05% respectively. Similarly PAb against DME exhibited very low cross-reactivity to ME and isomiroestrol with 1.26% and 0.42% respectively. The linearity range of ELISAs for both ME and DME were 0.73 – 3,000 ng/ml, which the coefficient of variation (CV) were less than 5%. The percentages of recovery were 98.80 – 104.37% and 99.82 – 102.58% for ME and DME, respectively in *P. candollei* samples Validated ELISAs were comparable with HPLC method in samples with high ME and DME contents, which coefficient of determination were 0.9996 and 0.9993, respectively. In additional, ELISAs could analyze in the samples undetectable by HPLC. The ELISAs was applied for ME and DME determination in *Pueraria* spp. The developed ELISAs were high performance for ME and DME determination, which could be applied for *P. candollei* involved researches of pharmacology and dosage form development. With simple and inexpensive procedure of ELISA, it could be applied for raw material and final product quality control during production process of industrial level.

PK49 Influence of different solvents on extractable saponins from Rusci rhizoma

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Butcher's broom (Rusci rhizoma, *Ruscus aculeatus* L., Ruscaceae) is traditionally used against chronic venous disorders. Beside phenolic compounds, especially furo- and spirostanol saponins seem to play an important role in the efficacy of the drug. Recent results indicate that furostanol saponins with a C-22 methyl ether are probably artifacts caused by methanol used in the isolation procedure or as component in the mobile phase of an HPLC system. Aim of the present study was to investigate the influence of different extraction solvents on the yield of saponins and artificial C-22 ethers. Powdered Rusci rhizoma was extracted with MeOH, EtOH, 1-Prop, *i*-Prop and 1-BuOH. The C-22 methyl, ethyl, and 1-propyl ethers could be detected in the respective extracts using HPLC-MS with a H_2O-MeCN gradient. No butyl and *i*-propyl derivatives could be observed. The amount of saponins in all extracts was examined by ^{1}H-NMR calculating the ratio of the saponin signal H-6 and the signal of the internal standard phloroglucinol. The lowest saponin concentration was determined in the MeOH extract. The 1-Prop, 1-BuOH, and *i*-Prop extracts showed higher and similar saponin concentrations compared to the MeOH extract (around 1.5fold), whereas the highest concentration was determined in the EtOH extract (1.72fold). As no C-22 artifacts could be detected and an acceptable yield could be achieved, 1-BuOH or *i*-Prop are recommended as extraction solvents for saponins from Butcher's broom.

L. Herbal medicinal products in animal healthcare and veterinary medicine

PL1 Complement fixing activity and *in vitro* inhibition of cytochrome P450 3A4 by *Aronia melanocarpa* constituents

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Extracts, subfractions, isolated anthocyanins and procyanidins, and two phenolic acids from aronia [*Aronia melanocarpa*] were investigated for their CYP3A4 inhibitory effects, using midazolam as the probe substrate and recombinant insect cell microsomes expressing CYP3A4 as the enzyme source. Procyanidin B5 was a considerably stronger CYP3A4 inhibitor *in vitro* than the isomeric procyanidin B2 and comparable to bergamottin, a known CYP3A4 inhibitor from grapefruit juice. The inhibitory activity of proanthocyanidin-containing fractions was correlated to the degree of polymerization. Among the anthocyanins, cyanidin 3-arabinoside showed stronger CYP3A4 inhibition than cyanidin 3-galactoside and cyanidin 3-glucoside. Thus, the ability to inhibit CYP3A4 *in vitro* seems to be influenced by the sugar unit linked to the anthocyanidin. Also, the extracts, subfractions, isolated anthocyanins and procyanidins together with isolated pectic polysaccharide fractions were analysed for complement fixing activity. Most of the polyphenols showed higher complement fixing activity than the polysaccharide fractions. Among the anthocyanins, cyanidin 3-glucoside possessed the highest activity, and of the isolated procyanidins B2, B5 and C1, the C1 showed the strongest and B2 the weakest complement fixing activity.

PL2 Sainfoin production and contents of condensed tannins in the leaves

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Sainfoin (*Onobrychis viciifolia* Scop.) is a forage legume of drier regions. Due to its anthelmintic properties the sainfoin is a natural alternative to drugs to control parasitism in the guts of small ruminants. Recent parasitological experiments on sheep and goats suggest that moderate dietary concentrations of condensed tannins (CT) can beneficially affect their health and performance. The effectiveness of the use of this feed seems very closely related to a minimum content of CT in the leaves. The literature reflects the variability of CT observed under the conditions of production. The studies conducted by Mediplant, in Switzerland from 2010 to 2012, have clarified the importance of the cultivar, harvest frequency and harvest stage on the CT content. A harvest at the early flowering stage offers the best guarantee to get high levels of TC (up to 6 – 7%), especially for the first harvest in spring. During the season the content of CT content is increasing from spring to autumn. The choice of the adapted cultivars (Perly, Perdix) is also crucial for content of CT in leaves (4 – 7%), but also for the productivity and the sustainability of the crop.

PL3 Phytochemical variability of common tansy

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Common tansy (*Tanacetum vulgare* L.) is a perennial, herbaceous flowering plant of the aster family, native to temperate Europe and Asia. Common tansy is often mentioned in the literature as a plant with de-worming properties of livestock (Waller et al., 2011). Its use in commercial products is also reported (Valchev et al., 2009). However, only few studies have been devoted to the domestication and cultivation of this perennial species. During three years (2004 – 2006), a study was conducted in Switzerland to analyse the morphological and phytochemical variability present within the Asteraceae species. Thirty accessions from ten countries were evaluated. Significant differences between the accessions were recorded, such as the essential oil content of the leaves and flowers (0.30 – 1.39%) and high variations of the contents of several

molecules in the essential oil such as α-thujone (0 – 84%), β-thujone (0 – 96%), chrysanthenone (10 – 83%), lyratol (23 – 55%) and umbellulone (6 – 36%). This high variability, especially the composition of the essential oil, is a valuable basis for a breeding program. Important knowledge was also gained for breeding and cultivation of common tansy (floral biology, harvesting stage, pests, yields, location). **References:** [1] Waller P.J., Bernes G., Thamsborg S.M., Sukura A., Richter S.H., Ingebrigsten K., Höglund J., 2001. Plants as de-worming agents of livestock in the nordic countries: historicol perspective, popular beliefs and prospects for the future. Acta Vet. Scand., 42, 31 – 44. [2] Valchev G., Popova-Ralcheva S., Bonovska M., Zaprianova I., Gudev D., 2009. Effect of dietary supplements of herb extracts on performance in growing pigs. Biotechnology in Animal Husbandry, 25(5/6), 859 – 870.

PL4 The oregano cultivar CARVA, a security for the supply of natural carvacrol

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The last two decades have seen a substantial increase in the use of aromatics herbs and essential oils for animal health and nutrition (Franz et al., 2010). In relation to public awareness of the potential health risks and environmental problems caused by in-feed antibiotics, growth hormones and some synthetic pharmaceuticals, as well as in relation with trends towards more natural approaches of food production, numerous research programs were established focusing on cultivation and extraction of plants and the use of their health related compounds for feeding animals. Oregano (*Origanum vulgare* L.) and especially its essential oil rich in carvacrol is a very promising plant with a high potential for animal health and nutrition. The production of carvacrol through the cultivation of oregano offers a high value natural product with a high supply security, a high quality and a good traceability. The oregano cultivar CARVA, developped by the Swiss Reseach Station Agroscope, answers perfectly to the demand of the molecule carvacrol and assure high and stable yields with a low variability of quality during the year and over the years. The contents of essential oil (6 – 7%) and carvacrol (75%) are very high as shown by several tests conducted in different regions of middle Europe. With a potential production within two years of 400 l/ha of essential oil with a carvacrol content of 75%, oregano cultivation with the cultivar CARVA can be considered as a valuable source of natural carvacrol for the industry. **References:** [1] Franz C., Baser K.H.C., Windisch W., 2010. Essential oils and aromatic plants in animal feeding-a European perspective. A review. Flavour Fragr. J., 25, 327 – 340.

PL6 Effects of herbal products *in vitro* and *in vivo*

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In the framework of a project for the Dutch Government on "Quality and safety of herbal products for production animals" the antimicrobial action of 23 products was investigated both by microbroth dilution and agar diffusion tests. Bacteria tested were *Salmonella typhimurium*, *Staphylococcus aureus Hoechst*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli* ATCC 11303, *Escherichia coli* "Bay" and *Enterococcus faecalis*. The products were tested with and without buffer, because products with a low pH may give growth inhibition which is not related to the herbs. In some products there was a marked difference between the microbroth dilution test and the agar test and also in many products there were differences between the results with and without buffer. Some products showed very good antimicrobial action whereas others did not show any activity. To examine the effects *in vivo* five of these products (both active and non-active, table 1) were fed to broiler chicken which were fed a nutritional sufficient diet containing a high amount of wheat which may impair digestion and gut health.

Tab. 1: Main ingredients herbal products

Product	Mean ingredients
Negative control	none
Biostrong 510	Thyme oil and star anise oil, bitter substances, pungent substances and saponins
Bronch Arom	Anise oil, thyme oil, eucalyptus oil
Allimax	Garlic
Duo Kruidenelixer	120 herbs: a.o. sage, rosemary, thyme, devils claw
PrimeFulvic	Fulvic acid

The products were compared to a control group and the trial was designed as a randomized complete block consisting of six repetitions per treatment. Data on growth, feed conversion and weight gain were collected and the jejunum was sampled for histological investigation. Villus crypt ratio was determined as an indication for gut health. Three of five herbal products showed significant differences as compared to the control group, two products with superior weight gain and one product with reduced weight gain (table 2). The two products which showed the best results in the broilers were products that showed almost no antimicrobial action *in vitro*. We concluded that in this experiment effects on gut health in broilers was not related to antimicrobial action.

Tab. 2: Body weight gain (BWG; g), feed intake (FI; g), and feed conversion ratio (FCR; g/g) from D 0 – 35, as affected treatment.

Treatment	BWG		FI	FRC	
Negative control	2547	ab	3771	1.481	a
Biostrong 510	2528	ab	3767	1.491	ab
Bronch Arom	2467	a	3734	1.514	b
Allimax	2546	ab	3758	1.476	a
Duo Kruidenelixer	2615	b	3860	1.477	a
PrimeFulvic	2578	b	3844	1.49	ab

PL7 Potential of alternative forage plants (herbs and legumes) in terms of secondary plant metabolites and capability of protein precipitation

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High contents of rapidly rumen degradable protein in forage plants are associated with metabolic and hepatic stress, disorder of proliferation and poor N utilization. Phytogenic agents, particularly tannins, may favorably modify rumen protein degradation and improve animal health and productivity (Mueller-Harvey 2006). To identify home-grown forage plants of such properties, freeze-dried and grounded material of six herbs and nine legumes (table 1) harvested in 2010, was analyzed in terms of condensed tannins (CT), total phenolics (TP) and the ability to precipitate the model protein bovine serum albumin (BSA). In addition, crude protein was fractionated according to the Cornell Net Carbohydrate and Protein System. It was assumed that plants with decent CT concentrations are able to form complexes with proteins, which consequently is reflected in protein quality. As expected, CT and TP concentrations as well as protein precipitation capacities were highest in *Onobrychis viciifolia* Scop. (Ov) and *Lotus corniculatus* L. (Lc) (table 1). *Sanguisorba minor* Scop. (Sm) with negligible CT but highest TP concentration showed above-average protein precipitation capacities (table1).

Tab. 1: CT, TP and capability to precipitate BSA of all evaluated species

Species	Common name	CT2 (% DM)		TP (% DM)		BSA precipitation (mg/g plantDM^{-1})	
Carum carvi L.	Carraway	0.04	-	4.44		1.05	-
Lolium perenne L.	Perennial ryegrass	0.04	-	1.58	-	0.03	-
Galega orientalis L.	Goats rue	0.05	-	3.98	-	1.09	-
Melilotus officinalis L.	Yellow sweet clover	0.06	-	1 59	-	2.15	-
Melilotus alba Medik.	White sweet clover	0.06	-	1.67	-	0.98	-
Taraxacum officinale Wiggers	Dandelion	0.07	-	3.54	-	2.81	-
Achillea millefolium L.	Yarrow	0.07	-	9.45		0.99	-
Medicago sativa L.	Alfalfa	0.08	-	1.37	-	0.41	-
Medicago lupulina L	Black medic	0.09	-	1.62	-	0.57	-
Cichorium intybusL.	Chicory	0.09	-	6.14		0.96	-
Trifolium repens L	White clover	0.11	-	1.80	-	0.31	-
Trifolium pratense L.	Red clover	0.11	-	3.14		0.64	-
Trifolium hybridum L	Alsike clover	0.12	-	2.75	-	2.72	-
Sanguisorba minor Scop.	Salad burnet	0.17	-	18.56	+	28.15	+
Plantago lanceolata L.	Narrowleaf plantain	0.30	-	6.61	+	2.01	-
Lotus comiculatus L.	Birdsfoot trefoil	1.93	+	4.80		18.62	+
Onobrychis viciifolia Scop.	Sainfoin	8.39	+	10.33	+	125.44	+
Total average of all species		0.69		4.90		11.11	

+/-= statistical deviations from total average ($p < 0.001$)

As hypothesized, Ov contained statistically significant ($p \leq 0.01$) below-average amounts of rapidly rumen degradable fractions (A and B1) and high amounts of not degradable fraction C. As to fraction A and B1, Sm showed equal characteristics. Sm contained significant ($p \leq 0.001$) highest amounts of rumen-undegradable but intestinal available fraction B3. No anticipated distribution of protein fractions could be observed in Lc. Results demonstrate that CT concentration seems to be an insufficient criterion to identify forage plants that potentially effect ruminant's protein metabolism. However, transferability of findings to the animal in-vivo situation has to be examined. **References:** [1] Mueller-Harvey, I. 2006. J.Sci.Food Agric.86:2010 – 2037.

PL8 Administration of Silver fir (*Abies alba* Mill.) to goats and its potential to control gastro-intestinal parasites

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Gastrointestinal nematodes (GIN) substantially impact on goat health. Because of the widespread development of GIN, resistant to allopathic drugs, further research into alternative parasite control methods is required. Silver fir, *Abies alba* Mill (SF), is traditionally used by Swiss farmers in order to control GIN infections of goats and to improve animal welfare. A survey among goat farmers revealed that they use SF in winter during a period of approximately five months. The estimated daily dose of SF per animal and day vary between 8 g to 600 g dry matter (DM). As there is scientific evidence neither for an anthelmintic potential of SF, nor on its influence on feed intake and milk performance of goats, we conducted a study with 30 animals. A 20 day feeding experiment was performed with 15 goats (group A), which were daily fed with SF in addition to their basic feed. The remaining 15 goats (group B) were fed with the basic ration only. Individual faecal egg counts (FEC) were conducted for all goats. Furthermore, the effects of SF on the intake of the basic feed, and on yield and composition of milk were analyzed. For 12 representative samples of SF we determined the content in total phenols (TP), and the composition of the essential oil (EO). SF contained 0.4 – 1.2% TP (0.9% TP on average) in fresh matter. Limonene, bornyl acetate and beta caryophyllene were identified as characteristic constituents of the EO. Administration of SF did not reduce GIN FEC significantly. The daily intake of SF per animal was 261 ± 0.22 g DM. Although SF reduced the basic feed intake significantly, SF significantly increased the total DM intake (A: 1948 ± 93 g DM; B: 1797 ± 93 g DM). SF had no effect on yield and composition of milk. Although farmers are convinced of the anthelmintic potential of SF, our short term study could not point to such an effect. Possibly a prolonged period of SF administration is necessary for such effects to become apparent.

PL9 Antimicrobial activity and stability of *Fraxinus rhynchophylla* Hance extract

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Clostridium perfringens is a gram-positive, spore-forming and anaerobic bacterium which is widely found in the food and intestinal tract of human and animal. *C. perfringens* can cause diarrhea and abdominal pain in human and necrotic enteritis, enterotoxemia and hamorrhagic gastroenteritis in animal due to several exotoxins of *C. perfringens*. e.g. A type α~toxin, B type α, β, ε and C type α, β. In this study, antimicrobial activity of *Fraxinus rhynchophylla* Hance extract against *C. perfringens* isolated from intestinal tract of broiler chickens and stability agaist heat and pH were investigated. Ethanol extract of *F. rhynchophylla* suppressed growth of *C. perfringens* at a concentration of 0.1 mg/mL (Fig. 1), whereas the growth of lactic acid bacteria, *Bifidobacterim bifidum*, was not affected by the extract. Moreover, growth of *B. bifidum* was stimulated about 5~16% compared with control after treated with the extract for 24~72 hours (Fig. 2). Antimicrobial activity of *F. rhynchophylla* extract was not altered after heated at 100 °C for 60 minutes and sustained in wide range of pH (3~11). These results suggested that *F. rhynchophylla* extract could be used as natural source for the development of antibiotics alternative.

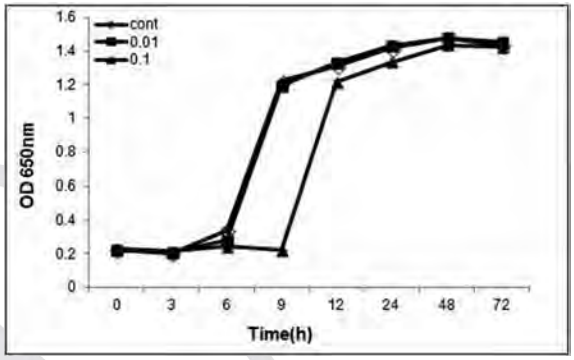

Fig. 1: Inhibitory effect of *F. rhynchophylla* extract on the growth of *C. perfringens*. *C. perfringens* was incubated at 37 °C for 0~72 hours under anaerobic condition, and the absorbance of broth was measured at 650 nm. cont: cultured without *F. rhynchophylla* extract, 0.01: treated with *F. rhynchophylla* extract at a concentration of 0.01 mg/mL, 0.1: treated with *F. rhynchophylla* extract at a concentration of 0.1 mg/mL

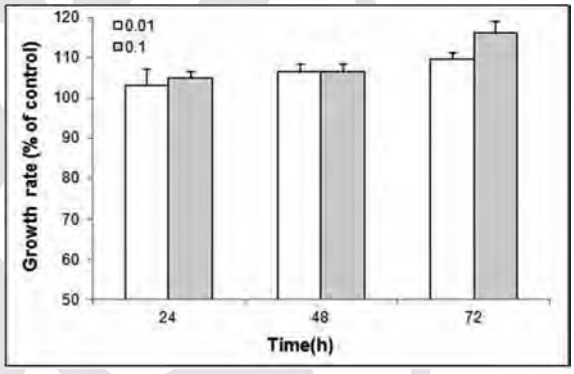

Fig. 2: Stimulatory effect of *F. rhynchophylla* extract on the growth of *B. bifidum*. *B. bifidum* was incubated at 37 °C for 24~72 hours under anaerobic condition, and the stimulatory activity of *F. rhynchophylla* extract was analyzed comparing with absorbance of non-treated broth medium (control). 0.01: treated with *F. rhynchophylla* extract at a concentration of 0.01 mg/mL, 0.1: treated with *F. rhynchophylla* extract at a concentration of 0.1 mg/mL. Means and standard errors are based on data from three replicates.

PL10 Antimicrobial activity of some phytochemical compounds against antibiotics resistant bacteria

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Plant secondary metabolites are important naturally occurring substances from renewable sources, which can be used for disinfections of animal farms. This research focuses on antimicrobial activities of plant compounds (phenols, flavonoids, essential oils) isolated and identified from species of *Lamium album* L., *Rosmarinus officinalis* L., *Monarda*

didyma L. and *Angelica archangelica* L. grown in Lithuania. The total amounts of phenolic compounds and total amounts of flavonoids were tested in the methanolic extracts of the plants. The essential oils were analyzed by gas chromatography – mass spectrometry (GC-MS). The study showed that the phytochemical composition of the plants investigated differs considerably. In our study, plants extracts were tested on their disinfecting effects against different bacterial species by determination the minimal inhibition concentrations (MIC). Investigated microorganisms were *Escherichia coli*, *Proteus vulgaris* and *Staphylococcus aureus* with and without antibiotic resistances. Isolates with antibiotic resistances originate from poultry farms and are known to occur even in air. Preliminary results show that the MIC range between 4 – 13% for the different extracts and bacterial species.

Tab. 1: Determination of MIC (%) of methanolic plant extracts: *Lamium album* L., *Rosmarinus officinalis* L., *Monarda didyma* L., *Angelica archangelica* L.

Microorganisms from livestock	*Lamium album* L.	*Rosmarinus officinalis* L.	*Monarda didyma* L.	*Angelica archangelica* L.
Escherichia coli	11	11	9	11
Escherichia coli (ESBL)	13	12	11	12
Staphylococcus aureus	11	9	8	9
Staphylococcus aureus (MRSA)	11	7	5	7
Proteus vulgaris	8	6	4	7
Proteus vulgaris (ESBL)	8	7	7	9

(ESBL – resistance to beta-lactam antibiotics; MRSA – methicillin resistance microorganisms)

PL11 Can traditionally administered home remedies be recommended? An assessment of 287 herbal home remedies for cattle, sheep, goats, pigs and horses, collected in the Swiss Canton of Graubuenden

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Introduction: In Switzerland, the administration of medicinal plants to livestock is almost completely confined to the use of home remedies. To save this traditional knowledge, which has been handed down through word of mouth, a project aims to collect this information from different regions of Switzerland. Grison home remedies have been evaluated to decide whether or not they can be recommended. **Methods:** 32 current or former Grison livestock keepers (average age 59 years) provided information on the home remedies they use or have used to maintain the health of their cattle, sheep, goats, pigs and horses and to treat them. The data collection was conducted by interview (28 on-site, 3 by phone, 1 in writing) with a standardised questionnaire about collecting medicinal plants, and preparing and administrating home remedies for farm animals. Only home remedies containing plants, lichens or products deriving from them were assessed by a practising veterinarian and a pharmacist.

Results: Grison livestock keepers mentioned 407 home remedies. The assessment included 287 herbal home remedies with 346 different indications. The investigations resulted in classification into 3 categories: (1) 278 indications that can be recommended, provided, in some cases, changes were made to either their preparation or administration. (2) 17 indications that could not be assessed and (3) 51 indications where their use is not recommended at all. Overall, 122 different, mainly herbal ingredients were used in the home remedies assessed. The most frequently applied herbal drugs are shown in the table, including their legal status for use in food-producing animals and the results of the assessment. **Discussion/Conclusion:** To enable livestock keepers to gain access to traditional knowledge, experts need to make recommendations for appropriate preparation and application of home remedies, to provide advice on necessary precautions and information on the legal situation for use on farm animals.

PL12 Application of *Cymbopogon winterianus* Jowitt and *Azadirachta indica* A Jussin the control of *Rhipicephalus* (*Boophilus*) *microplus*

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Parasitism constitutes one of the main problems that affect livestock. Commercial acaricides have been used to control tick, but these chemicals have been responsible for the development of drug resistance and residues in the environment. Changes in the concept of production determined the search for natural measures, which guarantees animal sanity along with safety of the produced food. This assay had as objective to evaluate the citronella oil and neem oil in the control of bovine ticks. By the technique of adult ticks immersion, 280 ticks were evaluated, distributed in equal number throughout four treatments: negative control group, positive control (ivermectin), neem oil and citronella oil. It was analyzed the mortality index, estimated reproduction, product efficiency, index of eggs production and the hatchability rate. The efficiency of the product was verified by the mortality index just for the positive control group (100%) and citronella oil (97.14%). Also, the citronella oil inhibited the eclosion of eggs in 100%. The other treatments did not presented the minimum eclosion inhibition level of 95%. Under the conditions of the present assay citronella oil was efficient against *Rhipicephalus (Boophilus) microplus*. This result was not observed in relation to the neem oil. Keywords: Agroecology, Citronella, Neem, Organic Production, Phytotherapy, Ticks.

PL13 Evaluation of antifungal activity of extracts of *Punica granatum* L. on *Malassezia pachydermatis*

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Fungal external otitis is a major diseases in small animals and the infection by *Malassezia pachydermatis* is the most frequent. The growing demand for medicinal plants is due to the indiscriminate use of antibiotics, and high recurrence rates. This work aimed to evaluate the aqueous and alcoholic extracts of *Punica granatum* L., at different concentrations, on *M. pachydermatis*. The fungus was isolated from a dog holder of chronic otitis and the diagnostic was confirming from imprint on glass slide and Gram stain. The suspension of *M. pachydermatis* was adjusted to concentration of 1×10^6 CFU/mL. The sensitivity of the microbial isolates was determined in quintuplicate by the technique of disc diffusion. The minimum inhibitory concentration was determined by inhibition zone greater than 15 mm. The aqueous extract was made by the cold extraction (24 hours), in water bath (60 minutes), boiling (10 minutes) and by infusion (60 minutes). The alcoholic extract was made

only by the cold extraction at different extraction times: 15, 30, 60 and 90 days. After obtaining the extracts at 50% (w/v), additional concentrations were prepared at 40, 30, 20 and 10%. The aqueous extract obtained by boiling was more efficient in inhibiting the growth of *M. pachydermatis*, proceeded by the extracts obtained by water bath, cold extraction and infusion. The extracts in concentrations of 50% regardless of the method used, showed greater zones of inhibition, however, all of aqueous extracts showed zone greater than 15 mm. Regarding the alcoholic extracts all of them presented inhibition of *M. pachydermatis*, except extracts at 10% and processed by 15 of extraction days. Thus, the results obtained by in vitro experiments, allows us to conclude that the extracts of *Punica granatum* L. presents potential clinical use for the control of otitis by *M. pachydermatis* in dogs.

PL14 Ability of eleven Thailand herbs to prevent silkworm fungal infections

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Thai silk, mainly produced in north and northeast of Thailand, is one of the natural textiles of the world most famous for its quality and beauty. Usually, microbial infection of the silkworm in rainy and summer seasons causes its lower productivity. Formaldehyde used by the farmers in its eradication is toxic to human and animals, as the contaminated fried pupa is a delicacy of some citizens. Hence, this research into alternative plant fungicides using soxhlet and maceration extracts (16 – 500 ppm) of eleven herb species of Thailand; *Ocimum tenuiflorum* L., *Mental cordifolia* Opiz., *Zingiber officinale Ros., Alpinia galanga* Swart, *Curcuma longa* L., *Cymbopogon citratus* (DC.) Staph., *Citrus hystrix* DC., *Zingiber montanum* Dietr., *Piper samentosum* Roxb., *Ocimum basilicum* Linn. and *Piper betle* Linn., three *Aspergillus species*; *A. flavus*, *A. oryzae* and *A. niger*, paper disc diffusion and microplate assays. Extracts of C. *hystrix, Z. montanum* and *C. longa* inhibited spore germination and growth of fungi, with MIC values of 6.25 – 125 ppm., while positive controls; toxic formaldehyde and ketoconazole showed MIC values of 3.12 – 6.25 ppm, suggesting their use as alternative to toxic formaldehyde. Thus, plant active compounds were investigated, other silkworm pathogenic microorganisms were also tested and field experiments are future study.

PL15 Antimicrobial activity of Romanian propolis ethanolic extracts against *E. coli* isolated from bovine mastitis

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Escherichia coli, nominated as one of the major bacterial pathogen of bovine mastitis, is also reported for its elevated antimicrobial resistance level [1,2]. The stringent need of research into natural alternatives to antimicrobials is emphasized as well by the current farm animal health and welfare policies [3] and propolis extracts were suggested by several authors for the anti-staphylococcal potency [2,4]. The study was aimed to investigate the antimicrobial activity of ten Romanian propolis samples against *E. coli* strains (n = 5) isolated from bovine mastitis; these strains were selected based on their resistance pattern: penicillin, streptomycin, tetracycline, novobiocin, neomycin, ampicillin, and amoxicillin-clavulanic acid (antimicrobials commonly used for mastitis treatment and control). As the first screening performed by a disk diffusion method indicated only a moderate activity in case of two propolis samples (with the maximum obtained inhibition diameters of 7 – 10 mm), minimum inhibitory concentrations (MIC) were established using a broth microdilution assay for propolis samples tested alone and with the amoxicillin-clavulanic acid combination. The most intense synergistic effect was noticed for the MIC of 8% v/v in case of propolis sample with the highest concentration of total phenolics (48.11 ± 2.76 mg/g propolis). Based on these *in vitro* results, Romanian propolis samples can be considered as complex natural antimicrobial products with beneficial effects on maintaining the bovine udder health and productivity when confronted with multiresistant *E. coli* strains.

Propolis Sample ID	Total phenolics (mg/g propolis) (Folin Ciocalteu method)	Diameter of inhibition zones (mm) (disk diffusion assay)	MIC (% v/v) (broth microdilution assay)	
			Propolis Sample	Propolis Sample + AMC
1 – 2	30.61 ± 1.16	7 ± 0.5	8	4
3 – 7	34.96 ± 9.36	10 ± 0.2	0.5	0.25
8 – 9	45.6 ± 3.27	21 ± 2.4	0.125	< 0.125
10	48.11 ± 2.76	22 ± 0.5	0.125	< 0.125
(AMC = amoxicillin-clavulanic acid)				

References: [1] Santana, H.F. et al. (2012) World J Microbiol Biotechnol. 28(2):485 – 91. [2] Pinto, M.S. et al. (2001) Braz. J. Vet. Res. Anim. Sci. 38(6):278 – 283. [3] Ivemeyer, S et al. (2011) Proceedings of the international mastitis conference, Wageningen Academic Publishers, 69 – 76. [4] Rahman, M.M. et al. (2010) Afr. J. Microbiol. Res. 4:1872 – 1878

PL16 Antimicrobial activities of honey bee venom against pathogens isolated from clinical bovine mastitis in Korea

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In Korea, honey bee (Apis mellifera, L) venom therapy (apitherapy) has been elucidated therapeutic value for farm animals with bacterial diseases and reported to be as effective as antibacterial drugs. This study was performed by using three honey bee venom products and 44 strains of bacteria isolated from infected cows' mammary quarters with mastitis in Korea. The concentration of major active components of bee venom were analyzed by HPLC and antimicrobial activity and potent were confirmed and compared among the three bee venom products by Cylinder agar plate method and Minimum Inhibitory Concentration (MIC). Time kill assays and electron scanning microscopy were used for observation of pore forming on cell membrane during the bacterial inhibition of the sensitive strains over time. In this study, the concentration of three major active components had a little frustration among three venom products, but the antimicrobial activities were not different statistically. All three products effectively inhibited the growth of *Staphylococcus aureus* (10 strains), Coagulase-negative *Staphylococcus* (CNS, 7 strains), and partially inhibited that of *E. coli* (2 of 7 strains) while didn't inhibit those of the others (at the over 500μg/ml of concentrations). MIC of *S. aureus,* CNS and *E. coli* were 62.5~125, 62.5~250 and 62.5~250 μg/ml respectively. However the pore forming on cell membrane wasn't observed by electron scanning microscopy at the time kill assay for the sensitive strains. It is needed to undergo more experimental investigation to ascertain the mechanism of action of antimicrobial activity of bee venom.

PL17 Impact of feeding mixtures of herbs on parasitic parameters in small ruminants

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Introduction: Commercial available mixtures of herbs promise a wide range of effects wether more „stress resistance" or „natural deworming". Material and methods: Three commercial mixtures of herbs were used in the additional feeding of ewes and goats in three farms: The powdery herbs P (garlic, turmic, himalayan cedar, ginger, long pepper) was fed daily for three weeks (8 g/day/animal) to sheep on farm 1 and to goats on farm 3. The pelleted herbs V (garlic, slippery elm, peppermint, thyme, cleavers, cinnamon, common nettle, quassia root) was fed daily for one week (10 g/day/animal) to sheep on farm 1 and the herbal extract herbs A (garlic, mugwort, walnut, clove) was fed daily for one week (20 ml/day/animal) to sheep on farm 2. In farm 1 three month after feeding herbs P to the trial group, sheep were split up again to trial and control group to feed herbs V. Herbs V feeding in farm 1 was monthly repeated twice, herbs A feeding in farm 2 was repeated monthly once. Fecal egg count (fec) was recorded just before additional feeding of the herbs

started (week 0). Further fecal egg counts were conducted in regulary monthly intervalls (goats only once). All animals were on pasture during the whole grazing season. **Results:** In farm 1 no differences could be recorded wether between control and herbs P nor between control and herbs V. Sheep started with moderate fec and 12 weeks later had low fec. Fec in herbs V was numerically lower in herbs V than in control group. In farm 2 fec in herbs A was significant lower in trial group only after the first feeding periode. No differnces could be seen in farm 3 between control and trial group of the goats. **Conclusions:** The additional feeding of herbal mixtures following producers guidelines under practical farming conditions could not present any lasting effect on fecal egg ouptut in sheep and goats. More examinations are necesarry to verify effects to animals at least how "stress resistance" can be verified.

PL18 Additional feeding of Asa foetida to sheep infested with *Haemonchus contortus*: Evaluation of fecal egg count and haematocrit

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Introduction: Asa foetida, the resinous product extracted of Ferula assa-foetida L., is used as a remedy in the central region of Asia in diseases including endoparasitism in humans. In this trial powdery Asa foetida was additionally fed to sheep, infected with *Haemonchus contortus*, to evaluate fecal egg count and haematocrit. Material and methods: 17 endoparasite free sheep, were assigned to control group (n = 5), group 1 simple concentration (n = 6) and group 2 double concentration (n = 6). The sheep were infected with 3000 larvae III of *Haemonchus contortus*. 4 weeks after infection group 1 was fed 0,08 g Asa foetida 5-fold concentrate solved in 10 ml water per animal per day and group 2 was fed twice of the amount (0,16 g). Control group received 10 ml water only. Asa foetida was fed daily over a period of three weeks. Fecal egg count and haematocrit were performed weekly. The powdery Asa foetida was bought in a pharmacy. The trial was approved by the government (Ges-140378/6 – 2012-Hi). **Results:** In fecal egg count no effect could be detected. At the end of the observation period fecal egg count was reduced in all groups but most in group 2. In haematocrit no statistical differences were found between groups but haematocrit in Asa foetida groups were higher during the whole examination period. **Conclusions:** The additional feeding of two concentrations of Asa foetida to sheep, infected with *Haemonchus contortus*, confirmed no effect on fecal egg count and haematocrit. The missing effect could be connected with low amounts of sulfur compounds (7.6 ppm *E*-n-propenyl-*s*-butyl-disulfide and 4.6 ppm *Z*-n-propenyl-*s*-butyl-disulfide). Other samples of Asa foetida from Afghanistan showed much higher amounts of these compounds (more than 3000 mg/kg) but pharmacopeia quality was not ensured. More information and examination are necessary in pharmacologically active constituents in different species of Ferula assa foetida to be recommended in additional feeding for endoparasite control.

PL19 Effects of feeding mixtures of herbs on coccidial shedding and weight gain in lambs

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Introduction: An additional feeding based on herbs was tested in lambs for its efficacy against shedding of Eimeria spp. and weight gain. Material and methods: Three mixtures of herbs were used: powdry herbs P (garlic, turmic, himalayan cedar, ginger, long pepper), pelleted herbs V (garlic, slippery elm, peppermint, thyme, cleavers, cinnamon, common nettle, quassia root), liquid extract herbs A (garlic, mugwort, walnut, clove). Trial 1: The lambs in the sheep farm were fed a pelletized lamb feed concentrate from the first day of life until the time of weaning at the age of 8 weeks (19 kg bodyweight) in a creep feed. Lambs not fullfilling this weight criterion were weaned 4 weeks later (week 12). The herbs P was mixed into the concentrate for the trial group (4 kg/ton). Examinations for coccidial count and weight gain were conducted at 4 weeks of age, at weaning and 4 weeks after weaning. Trial 2: 24 already weaned and fattening lambs with an age of 17 weeks were allocated to 4 groups (herbs P, herbs V, herbs A, control). The lambs were on the pasture every day for 5 hours. In the stable hay and 300 g of barley grain was fed. The additional feeding of herbs took place with the concentrate. Herbs P were fed daily in the beginning for three weeks (8 g/day/animal). Herbs V (10 g/day/animal) and herbs A (20 ml/day/animal) were fed daily for a week in the beginning and 4 weeks later for another week. Weight gain and coccidial count were conducted weekly. **Results:** Trial 1: Especially the lambs weaned with an age of 12 weeks had significant better weight gain than the control lambs. In trial 2 no statistical differences were found in weight gain. No long-lasting differences in coccidial count were found in both trials. **Conclusions:** Feeding herbs over a longer period of time had positive effects on production parameters. More information about duration of additional feeding and time of application during special events (parturition, weaning, etc.) would be usefull.

PL20 The *Ginkgo biloba* special extract EGb 761® improves behavioral activity and cerebral blood flow in aged Beagle dogs with mild cognitive dysfunction

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Aging in dogs, like in humans, is frequently accompanied by neurodegenerative processes associated with progressively increasing cognitive impairment. These disturbances are now considered to represent a discrete geriatric disorder called canine cognitive dysfunction syndrome (CCD). The main symptoms of CCD are an altered sleep-wake cycle, reduced social interaction, changes in residential behavior, disorientation, and reduced activity. Pathological changes associated with these cognitive deficits include amyloid deposits in the brain, changes in cerebral blood flow, glucose metabolism, and loss of white matter. Previously, EGb 761® has been shown to exert beneficial effects in treating disorders associated with impaired cognitive function. Thus, it was the aim of the present study to examine the influence of EGb 761® on behavioral activity as well as cerebral and peripheral blood flow in aged (> 8.75 years) male and female Beagle dogs (N = 8) with mild cognitive dysfunction. The animals received EGb 761® at oral doses of 1.5, 3, 10 and 30 mg/kg for each 6 days with intermittent placebo phases in between. Blood flow in the middle cerebral, carotid and femoral artery was measured by ultrasound while behavioral activity was monitored with the use of the Actiwatch method. The study demonstrated a dose-related response of the cerebral vessels that lasted for at least six days. Dilation of cerebral vessels and related increase in blood flow was most pronounced at doses of 3 and 10 mg/kg. Similarly, EGb 761® caused an increase in activity that peaked at a dose of 3 mg/kg with a declining effect at higher doses. A significant effect on peripheral blood flow was not observed. The results indicate that EGb 761® provides a safe alternative for the treatment of dogs with CCD, which may produce cardiovascular benefits. In addition, the data support the therapeutic application of EGb 761® in the treatment of cerebral circulatory complaints as well as cognitive disorders in humans.

PL21 Effects of *Moringa oleifera* Lam. dietary seed protein extracts on growth, nutrient utilization and blood parameters in common carp (*Cyprinus carpio*, L.) and Nile tilapia (*Oreochromis niloticus*, L.)

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The drumstick tree, *Moringa oleifera*, is a multi-purpose plant showing several beneficial effects, depending on plant part origin. The seeds for example show potential anti-biotic activity against certain pathogenic bacteria species. Furthermore they can be used for water purification since they contain specific proteins with coagulation properties. In these experiments we tested the effects of dietary *M. oleifera* seed protein extracts (MPE) on growth performance, nutrient utilization and blood parameters of the common carp, *Cyprinus carpio*, and on Nile tilapia, *Oreochromis niloticus*, two of the most important freshwater aquaculture fish species. The MPE was added in three concentrations (carp: 200, 400 and 600 ppm; tilapia: 400, 800 1200 ppm) to the diets and fed, beside a

negative control for eight weeks. During this period carp grew from initially 25.1 g to final body masses of 95.9 g (control), 97.2 g (200 ppm), 100.2 g (400 ppm) and 100.3 g (600 ppm) and tilapia from initially 5.49 g to final body masses of 46.6 g (control), 48.0 g (400 ppm), 42.4 g (800 ppm) and 49.4 g (1200 ppm). The feed conversion was best for carp fed with 400 and 600 ppm MPE (1.08 kg feed/kg body mass gain) and for tilapia fed 400 ppm MPE (0.84 kg feed/kg body mass gain). the best protein conversions in carp was achieved by the 600 ppm treatment (2.40 kg body mass gain/kg protein fed) while in tilapia the 400 ppm MPE treated group showed the highest protein conversion (3.00 kg body mass gain/kg protein fed). Higher supplementation levels with MPE resulted in higher red blood cell counts (RBC) in both species accompanied by increased hemoglobin concentrations and hematocrit in tilapia but not carp and in higher white blood cell counts in carp but not in tilapia. These results show that seed protein extracts from *Moringa oleifera* have potential as growth promoters in two of the world's most important freshwater aquaculture species by improving growth and nutrient utilization.

PL22 Milk fatty acids in dairy cows supplemented with larch (*Larix decidua* L.) sawdust

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Larch (*Larix decidua* L., Pinaceae) wood is known to contain flavonols (mainly dihydroquercetin and dihydrokaempferol) which possess a high antioxidant activity comparable to that of α-tocopherol. This study was conducted to investigate whether larch sawdust (byproduct of larch wood production), supplemented to dairy cows, have effects on milk fatty acids composition and on the oxidative stability of milk. Sixteen Italian Friesian dairy cows in mid lactation were used in a 5-wk study. All animals were fed a total mixed diet. Animals were divided into two groups, one treated group (n = 8) received 300 g/d of larch sawdust [1] and one negative control group (n = 8). The treatment lasted for 21 d. Milk fatty acids was determined on samples collected on day 20 and on the same samples after storing at 4 °C for four days. Milk stored at 4 °C for four days were analyzed also for hexanal content. Milk yield was not affected by treatment. Total polyunsaturated fatty acids were higher in milk from cows fed with larch sawdust ($p < 0.05$) with an highest proportion of C20:3n-6 fatty acid ($p < 0.01$). The fatty acids in control milk after 4 days storage (4 °C) were more susceptible of oxidation. Milk from cows fed with larch sawdust was more resistant to fatty acids oxidation (PUFAs, $p < 0.05$). Despite its high polyunsaturated fatty acid content, milk from treated group was reasonably stable to oxidation with the lower hexanal production after 4 days of storage at 4 °C, than what observed in control milk ($p < 0.01$). These results suggest that larch sawdust is an interesting resource of valuable feed additive as naturally antioxidant. Moreover larch sawdust being a waste product, could represent an economically valid tool, keeping in mind that, the development of integrated biorefinery processes should be needed. Further studies with other animal species will be necessary to confirm the application. [1]provided by Jannach Lärchenholz GmbH, Thalheim, Austria-(SAFEWASTES, EU pr. 513949)

PL23 Case report: The chinese formula "Bai He Gu Jin Tang" with lily bulb relieves chronic respiratory disease in horses

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Chronic respiratory diseases (CRD) in horses are very challenging to treat. The underlying mechanisms are found in inappropriate immune responses either to previous infectious agents or to dust or pollen. There are two kinds of chronic respiratory diseases: chronic obstructive bronchitis with increased cellular immune components and asthma and asthma-like disease with an elevated humoral response. Clinical symptoms of CRD include chronic cough, often non-productive, and difficult breathing. In later stages there appears a heave line defined by the oblique abdominal muscles. Bai He Gu Jin Tang is a well known formula in Traditional Chinese Medicine used in the treatment of lung diseases. It is composed of lung-protective ingredients (table 1) and is an ideal formula in combating CRD in horses. Mick is a 15-year-old Connemara pony with intermediate to severe episodes of CRD during the last four years. He had been repeatedly treated with broncho-dilators and antibiotics with little or no effect. During winter symptoms worsened and he was prone to infections of the upper airways. His general condition and nutritional state were good, his mucous membranes were slightly red but there was not any nasal discharge. He presented a mild to intermediate respiratory sound of the lungs and slightly labored breathing during intermediate work. Mick was given the Chinese Formula "Bai He Gu Jin Tang" over the next 3 months and was closely monitored every 4 weeks. Over a period of 4 months the cough symptoms improved until they finally stopped. His breathing also improved remarkably and it was possible to work him on a normal level. However, close monitoring will have to be continued to prevent a relapse into his previous state.

Tab. 1: Ingredients and their percentages of the Chinese Formula *Bai He Gu Jin Tang*

Name	Pin Yin	Percentage
Lilii, bulbus	Bai He	16
Ophiopogonis, rad	Mai Men Dong	13
Rehmannia viride, rad	Sheng Di Huang	12
Rehmannia praep., rad	Shu Di Huang	12
Paeonia alba, rad	Bai Shao	8
Fritillariae cirrhosae, bulbus	Chuan Bei Mu	8
Platycodi, rad	Jie Geng	8
Scrophulariae, rad	Xuan Shen	7
Glycyrrhizae, rad	Gan Cao	4

PL24 Traditional homemade herbal remedies used by farmers of northern Switzerland to treat skin alterations and wounds in livestock

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Ethnoveterinary surveys are missing for wide areas of Europe. During the years 2011 and 2012 80 farmers on 64 farms in seven cantons of Northern Switzerland (Aargau, Zürich, Schaffhausen, St. Gallen, Thurgau, Appenzell Innerhoden and Appenzell Ausserrhoden) were interviewed. More than 500 homemade herbal remedies (HMHR) were documented regarding the used plant species, modes of preparation, dosage, routes of administration, category of use and origin of knowledge. A selection was made by choosing all HMHR which (a) contain only one herbal drug, (b) are used to treat skin alterations and wounds, (c) were administered to the skin, (d) were obtained from forefathers and relatives and (e) have been used by the interview partners themselves at least 5 times during the last five years. The two latter criteria were introduced to analyse only formulations with a high level of tradition. The 34 selected HMHR contained twelve plant species from 8 families. The most frequently used plant species were from the family of Asteraceae (Table 1), and flowers were the most often used plant parts. The processing of the herbs included mostly extraction with oil/fat or water, but also maceration with ethanol of varying percentage. In contrast, fresh Comfrey roots were grated and administered directly to the skin. The formulations where used in 49 different applications for treatment of wounds and other skin alterations in livestock, mainly in cattle. Whenever possible, the weight of the used plant was determined to calculate concentrations in g drug equivalent per 100 g of finished product. Most of the documented concentrations were in a lower range compared to literature. The uses of the most frequently named medicinal plants (chamomile, marigold and St. John's wort) can be regarded as well founded, considering recent pharmacological and clinical data. Other plants identified in this survey should be subject to further studies. (connect the author for references)

Tab. 1: Frequency of plant species in 34 homemade herbal remedies used by farmers of Northern Switzerland for 49 applications used to treat skin alterations and wounds in livestock

Description of 34 formulations				Route of administration of 49 applications			
No of formulations	family	botanical name	used plant part	coating	compress	washout	bath
7	Asteraceae	*Calendula officinalis* L.	flower	6		3	
6	Asteraceae	*Matricaria recutita* L.	flower		2	5	1
4	Hypericaceae	*Hypericum perforatum* L.	flower	6			1
4	Boraginaceae	*Symphytum officinale* L.	root	2	2		
3	Malvaceae	*Malva neglecta* Wallr.	herb		3		4
3	Apiaceae	*Sanicula europaea* L.	herb		1	1	1
2	Polygonaceae	*Rumex obtusifolius* L.	leave root	1			1
1	Asteraceae	*Arnica montana* L.	flower			1	
1	Chenopodiaceae	*Chenopodium bonus-henricus* L.	leave	1			
1	Malvaceae	*Malva sylvestris* L.	herb			1	
1	Pinaceae	*Picea abies* (L.) H. Karst.	excretion	4			
1	Asteraceae	*Solidago virgaurea* L.	no answer	2			

PL25

A herbal feed additive shows potential to improve metabolic situation in early lactating dairy cows

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The gap between performance and feed intake in early lactating dairy cows often leads to metabolic imbalance which is connected to udder inflammation (UI). A study with 72 dairy cows calving from November 2010 to March 2011 on 10 Swiss and German farms was conducted to test the effect of a herbal feed additive (HFA) containing mainly *Urtica dioica* L. (herba), *Silybum marianum* (L.) Gaert. (fructus), *Artemisia absinthium* L. (herba) and *Achillea millefolium* L. (herba). Cows were stratified (farm and milk yield) randomised divided into three groups. From 14 days prior predicted calving to the end of the following lactation cows received daily 100 g pellets containing **A**: 100% HFA, **B**: 50% HFA and 50% alfalfa and **C**: 100% alfalfa (placebo). Two or three cows per group were included per farm. Farmers documented the pellet intake individually per cow on a daily base. Cows with an intake less than two third of the offered dose per lactation part (early: day 1 – 100; mid: day 101 – 200; late: day 201 – 300) were excluded from analyse (A: 6 cows, B: 4 cows, C: 0 cows).

Tab. 1: Health and performance parameters of dairy cows getting two different amounts of a herbal feed additive [A: 100 g per cow and day; B: 50 g per cow and day] and placebo [C] respectively

	Feeding groups			significance
	A	B	C	
Milk aceton more than 3 times higher than 10 mg/dl in weekly milk samples during the first 10 weeks of lactation (% of cows)	**0**	**20**	**30**	**p = 0.047**
Average of the first 3 monthly milk recordings of the lactation				
daily milk yield (kg)	34.5 (± 12)	35.0 (± 11)	34.6 (± 12)	n. s.
milk fat (%)	3.9 (± 0.5)	3.9 (± 0.6)	3.9 (± 0.4)	n. s.
milk protein (%)	3.2 (± 0.06)	3.2 (± 0.06)	3.1 (± 0.05)	n. s.
milk urea mg/dl	22 (± 8)	22 (± 9)	22 (± 9)	n. s.
Somatic cell score				
1. milk recording of lactation	2.0 (± 1.4)	2.5 (± 2.3)	2.5 (+ 1.8)	n. s.
2. milk recording of lactation	**1.4 (± 1.9)**	**2.2 (± 2.2)**	**3.1 (± 1.8)**	**p = 0.036**
3. milk recording of lactation	1.8 (± 2.0)	2.3 (± 1.5)	2.7 (± 2.2)	n. s.
BCS lost > 0.5 during early lactation (% of cows)	38	40	48	n. s.
BCS lost: Lost of body condition score (BCS) compared to the BCS of the dry period				

Weekly milk samples from a healthy udder quarter were taken in lactation week 1 – 10 to analyse the aceton content indicating metabolic imbalance. Milk recording data (milk yield, milk contents and somatic cell score as UI marker) as well as the development of the body condition score, treatment, intercalving period and culling rate were analysed. Table 1 shows the results of early lactation, no further significant differences were found in mid and late lactation. Compared to placebo the feed additive A shows potential to improve metabolic situation in early lactation which is probably connected with a lower somatic cell score. The effects might be caused by appetising (*Artemisia absinthium L.* and *Achillea millefolium L.*) as well as liver protection (*Silybum marianum (L.) Gaert.*). Beside this *Urtica dioica L.* is documented from ethnoveterinary research to be used for restorative aspects. (contact the author for references)

M. Metabolic pathway engineering of secondary natural products

PM1

Metabolic engineering and elicitation of pharmaceutically active metabolites in *Rhazya stricta* (Apocynaceae)

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Rhazya stricta Decne., a small evergreen shrub native to the Middle East and Indian sub-continent, is a rich source of pharmacologically active terpenoid indole alkaloids (TIAs). The plant has been in the focus of phytochemical, pharmacological and ethnobotanical studies due to its long use in folk medicine to treat a variety of ailments. More recently especially the antimicrobial and anticancer properties of certain constituents (Fig. 1) have been established. TIAs are formed by the condensation of the indole and iridoid moieties catalysed by strictosidine synthase (STR) to form the precursor strictosidine. High STR activity associated with the induction of rate-limiting enzyme activities of the iridoid pathway (GES, G10 H) favours TIA production. Biosynthetic steps in the TIA pathway are co-ordinately regulated at the level of transcription of the structural genes by transcription factors (e. g. *orca3*). Wild type hairy root cultures were established in order to study TIA production and enable metabolic engineering. A validated GC-MS method was developed and 20 compounds were identified. Rhazinilam, rhazine and vallesiachotamine were present in several lines (Fig. 1). Gene(s) from the early part of the TIA pathway, single gene geraniol synthase (*ges*), geraniol 10-hydroxylase (*g10 h*) and strictosidine synthase (*str*) and double-gene (*str+orca3*), were introduced to *R. stricta* to investigate their overexpression effects on the TIA biosynthesis in transgenic hairy root lines. Integration and expression of the gene(s) were confirmed by molecular techniques. Suitable elicitations by methyl jasmonate to stimulate TIA accumulation were evaluated. Chemical analysis of transformed hairy roots and elicited samples showed an increased level of selected TIAs compared to the wild type hairy root lines and non-elicited samples. Further analysis revealed higher accumulation of total alkaloids in transformed and elicited hairy roots.

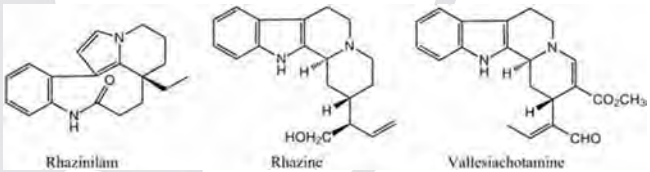

Fig. 1: Some anti-cancer compounds from *R. stricta*

PM2 LCMS Spectral Evidence of the Occurrence of Cannabinoid in *Cannabis sativa* Cell Cultures

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Cannabis sativa L. (marijuana; Cannabaceae) is a plant with worldwide distribution, yielding fiber and food, as well as a psychoactive drug. Cannabinoid and in particular the main psychoactive Δ^9-THC are promising substances for the development of new drugs and are of high therapeutic potential. *In vitro* production of cannabinoid using callus and cell cultures (CC) has not been reported yet [1]. We investigated the occurrence and accumulation of cannabinoid in CC of *C. sativa.* CC was established from leaf derived callus of *C. sativa* "bedrobinol" cultivar growing in Bedrocan BV Medicinal Cannabis, The Netherlands. The culture medium was modified Gamborg B5 [2] supplemented with different concentration of plant growth regulators; thidiazuron (TDZ), 6-benzylaminopurine (BA), and gibberellic acid (GA3) at 0.5, 1.0 or 1.5 mg/l. CC was kept on rotary shaker 120 rpm under permanent light at 27 ± 1 °C (Fig. 1). Growth parameters were measured weekly during 35 days of growth cycle. In this study, using LCMS we reported for the first time the accumulation of Δ^9-tetrahydrocannabinol (Δ^9-THC), tetrahydrocannabinolic acid (THCA), cannabinol (CBN), cannabigerol (CBG), cannabigerolic acid (CBGA), and cannabidiol (CBD) in the CC extracts. On the other hand, accumulation of cannabinoid was reduced after 21-days. The results indicate that our cell lines can be used as a model to investigate metabolic pathways of cannabinoid in *C. sativa. Keywords:* Cannabinoid, *Cannabis sativa*, Cell cultures, Gamborg medium, plant growth regulators, LCMS. **References:** [1] Pec J, Flores-Sanchez IJ, Choi YH, Verpoorte R (2010) Metabolic analysis of elicited cell suspension cultures of Cannabis sativa L. by (1)H-NMR spectroscopy. *Biotechnol Lett* 32 (7):935 – 941. [2] Gamborg OL, Miller RA, Ojima O. 1968. Nutrient requirements of suspension cultures of soybean root cell. *Exp. Cell Res.* 50: 151 – 158.

PM3 Rose hip special extract FB9440 inhibited osteoclastogenesis via NF-κB pathway

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Rosa canina L. is a medicinal plant largely used in traditional folk medicine. A systematic review to critically evaluate the evidence for the efficacy of complementary and alternative medicines in the management of osteoarthritis found some consistency to the evidence that rose hip may be effective. This might be due to anti-inflammatory and cartilage-protective properties. Osteoarthritis (OA) and osteoporosis (OP) are two common age-related disorders affecting quality of life of the elderly. Recent studies have revealed several factors which contribute to the pathogenesis of both disorders. These insights might contribute to the development of shared new treatment options in the near future. Increased subchondral bone loss is a characteristic feature of OP and the early stage of OA, and this finding is the rationale for studies on the effect of anti-osteoporotic drugs in OA. In addition, inflammation and a unfavorable body composition have been recognized as contributing factors for both disorders. Anti-resorptive drugs significantly reduce bone turnover, providing an increase in bone mineral density and a reduction in risk of fracture. Recent advances have identified the Receptor Activator for Nuclear Factor κB Ligand (RANKL) as a critical mediator of bone remodeling. RANKL is essential for the formation, function, and survival of the osteoclasts. It binds to its cognate receptor RANK on the surface of precursors and mature osteoclasts, and stimulates these cells to mature and resorb bone. Therefore we have studied the biological activities of an aqueous special extract from rose hip peels (DER 2 – 6:1, patent applied PCT/EP2008/068081), with respect to RANKL-induced osteoclastogenesis in RAW 264.7 cells. We show for the first time that rose hip extract FB9440 inhibited the activation of NF-κB mediated by RANKL and more importantly this extract showed a strong inhibition of RANKL-induced osteoclastogenesis even at low concentrations (10 µg/ml).

PM4 *In vivo* significance of tropinone reductases I and II in *Solanum tuberosum*

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Tropinone constitutes an important metabolite at the branching point of tropane alkaloid biosynthesis. Either tropinone is reduced to tropine by tropinone reductase I (TRI) leading to the formation of tropane alkaloids, or tropinone reductase II (TRII) forms the stereoisomer pseudotropine, precursor of calystegines. Potato (*Solanum tuberosum)* contains tropinone, accumulates calystegines but no tropane alkaloids. Nevertheless, the potato genome contains coding sequences for both kinds of tropinone reducing enzymes.

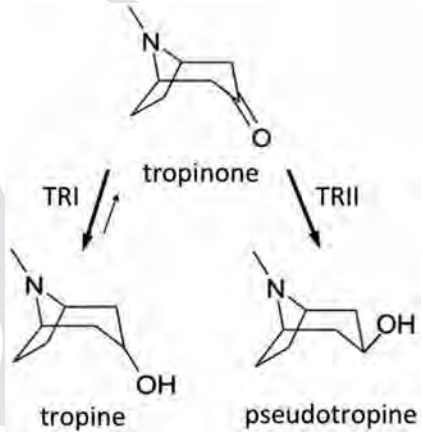

Fig. 1

The biological function of the *S. tuberosum* TRI (StTRI) is unknown. Although no tropine and derivatives could be detected, *StTRI* is expressed in some potato tissues. To elucidate the biological significance of this StTRI, RNAi and overexpressing potato plants were generated by agrobacteria transformation. *sttrI* knockdown and overexpression were evaluated by transcript analysis. Overexpression was confirmed by tropinone feeding and detection of enzyme activity. Furthermore, metabolite profiling analysis will help to gain insights into the biological function of the StTRI enzyme. Concurring with the calystegine accumulation, a gene coding for tropinone reductase II could be isolated from *Solanum tuberosum* before. The encoded enzyme StTRII is able to reduce tropinone to pseudotropine *in vitro* but its functional role in potato secondary metabolism was never proven. The *in vivo* significance will be studied in parallel to StTRI by RNAi of *sttrII* and analysis of calystegine content in calystegine accumulating plant tissue e.g. tuber sprouts.

PM5 Chemistry of Hermidin, the main alkaloid of the medicinal plant *Mercurialis perennis* L. (Euphorbiaceae)

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Piperidindione alkaloids are naturally occurring in different species of *Mercurialis* (Euphorbiaceae) [1]. Hermidin **1**, the main alkaloid of dog's mercury (*M. perennis*), is an air-sensitive molecule which is easily oxidized in aqueous solution to yield blue, yellow and yellow-brown compounds [1,2]. By LC-UV(DAD)-MS/MS analysis of an aqueous extract obtained from herbal parts of *M. perennis* the dicarboxylic acid **2** was identified for the first time. **2** is formed via a complex reaction pathway from **1** by a free radical mediated oxidation. This compound **2** (λ_{max} = 426 nm) is partly responsible for the typical orange-red color of fermented aqueous extracts from *M. perennis* which are applied in preparations for treating inflammatory events [3]. Furthermore, by extracting roots of *M. perennis* with aqueous acetone the novel constituent **3** was isolated as an artifact, which was proven by lack of this compound upon ethyl acetate extraction. After chromatographic purification the

structure of **3** was assigned by GC/MS, 1D- and 2D-NMR methods. Remarkably, **3** was also detected in fermented aqueous extracts of *M. perennis*, which was ascribed to trace formation of acetone upon fermentation. These findings may broaden our understanding of piperidindione alkaloid chemistry *ex vivo*. **References:** [1] P. Lorenz, J. Conrad, F. C. Stintzing (2012) *Chem. Biodivers.* 9: 282 – 297. [2] P. Lorenz, M. Hradecky, M. Berger, J. Bertrams, U. Meyer, F. C. Stintzing (2010) *Phytochem. Analysis* 21: 234 – 245. [3] P. Lorenz, C. Beckmann, J. Felenda, U. Meyer, F. C. Stintzing (2013) *Z. Phytother.* 34: 40 – 46.

PM6 Myth and ritual: The cardenolide pathway revisited

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The conception of the existence of well-defined biosynthetic pathways is outdated. This holds particularly true for the formation of small natural products (SNAPs). The catalytic efficiencies described for enzymes involved in the formation of SNAPS are amazingly low. Many enzymes have been shown to catalyze not only their reported "natural" reaction but also alternative reactions. In several cases, substrate as well as product promiscuity has been demonstrated. Cardenolide biosynthesis is a good example to explain how paradigms have to be messed up. According to the text books, early steps in cardenolide biosynthesis are supposed to be catalysed by 3β-hydroxysteroid dehydrogenase (3βHSD) and progesterone 5β-reductases (P5βR). When analysing 3βHSDs and P5βRs genes and enzymes in the genera *Digitalis*, *Erysimum* and *Arabidopsis* it became clear that small gene families exist that encode for 3βHSDs or P5βRs. Some of these genes can be induced by stress. All enzymes are substrate-promiscuous enzymes and are involved probably in more biosynthetic reactions than thought before. Several catalytically active 3βHSDs were found in *Erysimum* and in *Arabidopsis.* All of them catalysed the dehydrogenation and reduction of various C_{17}, C_{19} and C_{21} steroids. The enzyme also possesses steroid 17β-dehydrogenase activity but no Δ5 – 3-ketosteroid isomerase (3-KSI) activity as described for animal 3βHSDs. The existence of a discrete 3-ketosteroid isomerase was isolated from *Digitalis lanata.* Several individual P5βRs have been demonstrated to occur in *Digitalis, Erysimum, Arabidopsis* and other plant species. They all possess relaxed substrate specificities and reduce C = C double bond of various large and small 1,4-enones. Cardenolide-biosynthetic enzymes may also have other functions or are operative in other biosynthetic pathways. This has recently been demonstrated in iridoid formation.

PM7 VEP1- encoded enone 1,4-reductases from Brassicaceae: cloning, expression, molecular phylogeny and modelling

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Progesterone 5β-reductases (P5βR), which are members of the short chain dehydrogenases/reductases (SDR) superfamily are involved in the biosynthesis of 5β-cardenolides. These enzymes have been characterized as substrate-promiscuous enone 1,4-reductases and are encoded by Vein Patterning1-like (*VEP1*) genes, which occur in cardenolide producing plants as well as in non-cardenolide producing plants. Since cardenolides have been reported for a few Brassicaceae genera only, one may ask whether cardenolides originated several times in Brassicaceae or whether they were lost during evolution in most of the genera. In order to shed light on the conservation and/or the multiple evolution of genes coding for cardenolide-biosynthetic enzymes, we isolated P5βRs from several Brassicaceae. The nucleotide and deduced amino acid sequences of the new P5βRs were aligned with known and putative *VEP1*-encoded P5βRs. The new genes were over-expressed in *E. coli* and the respective P5βR proteins tested for their catalytic function. Molecular modelling was applied to elucidate structural and functional relationship between *VEP1*-encoded P5βRs.

PM8 Understanding adaptogens: new evidence on their possible effectiveness in stress-induced and ageing-associated disorders from a DNA microarray study of neuroglia cells

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Gene expression profiling was conducted on the human neuroglial cell line, after treatment with either the adaptogen, ADAPT-232, or its constituents, which included extracts of Eleutherococcus senticosus root, Schisandra chinensis berry, and Rhodiola rosea root, or several individual constituents, including eleutheroside E, schizandrin B, salidroside, triandrin, and tyrosol. All tested adaptogens had similar effects on G-protein-coupled receptor (GPCR)-mediated signal transduction through cAMP, phospholipase C, and phosphatidylinositol signaling pathways. Adaptogens may reduce cAMP levels in brain cells by downregulating the adenylate cyclase gene, ADC2Y, and upregulating the phosphodiestherase gene, PDE4D. This activity is essential for energy homeostasis and for switching between catabolic and anabolic states. All tested adaptogens upregulated the PLCB1 gene, which encodes phosphoinositide-specific phospholipase C and phosphatidylinositol 3-kinases, key players in the regulation of NF-kB-mediated defense responses. Other common targets of adaptogens included genes encoding the ERα estrogen receptor (up to 22.6-fold downregulation), cholesterol ester transfer protein (up to 10.6-fold downregulation), heat shock protein, Hsp70 (up to 45.0-fold upregulation), serpin peptidase inhibitor, and 5-HT3 serotonin receptor (up to 6.6-fold downregulation). These findings were consistent with the observed beneficial effects of adaptogens in stress-induced behavioral, mental, and ageing-associated disorders, including neurodegeneration, atherosclerosis, and impaired apoptosis. Panossian A, Hamm R, Kadioglu O, Wikman G, and Efferth T. (2013). Synergy and antagonism of active constituents of ADAPT-232 on transcriptional level of metabolic regulation of isolated neuroglial cells. Front. Neurosci.7:16. doi: 10.3389/fnins.2013.00016

PM9 Combining transcriptomics-based and proteomics-based approaches for functional characterization of terpene synthases of "Arnica da Serra" (Asteraceae)

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Lychnophora ericoides (Vernonieae: Asteraceae), known as "Arnica-da-serra", is restricted to Brazilian "Cerrado" and is used for wound healing. Its leaves produce a range of volatile sesquiterpenes original from the bisabolyl cations, also known as bisabolene-like derivatives. We have recently reported the bioactivity of essential oil fraction against invertebrate Acari (BALDIN et al., 2010) and the *in vitro* anti-hyper nociceptive ability of a major compound (PAVARINI, 2011). Enzymes behind the biosynthesis of these sesquiterpenes are usually the target of researches that are pushing the boundaries of natural products biosynthesis. This achievement often leans on the results of enzymes functional characterization. Our previous results were achieved through targeted-proteome searches by MALDI imaging highlight the perspective of protein mapping on plant tissues (PAVARINI, et al., 2012). Simultaneously, the biotechnology panorama is tied very tightly to fundamental sciences knowledge. Therefore, we are combining research fields at this present work in order to move towards a comprehensive description of these volatile sesquiterpenes biosynthesis pathway. Our efforts have been concentrated in the main subjects: (1) to achieve plant protein maps by using gel-based separation of protein extracts; (2) to reach protein identity information by spots digestion protocols, mass spectrometry and chemo informatics-based inventories; (3) to access transcripts encoding genes of terpene synthases; (4) to isolate the transcripts and amplify them; (5) to get these genes expressed using transfected microorganisms. We expect a bright outlook for generating combined molecular images of enzymes and metalolites using MALDI imaging to be settled at the end of this work. **References:** [1] Baldin, E.L.L. et al., Bolet. San. Veg. Plag., *36*, 125, 2010. [2] Pavarini, D.P. 126 p. Master's Disserta-

tion, FCFRP-USP, University Of São Paulo, 2011. [3] Pavarini, D.P. et al., Plant. Med., *78*, 1027, 2012.

PM10 Anti-inflammatory effects of lipids extract from the cod liver

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It is known that marine organisms contain a large spectrum of bioactive lipids including arachidonic acid and phospholipids, phosphatidic acid, polyunsaturated fatty acids (PUFA), epoxyeicosatrienoic acids, and others. Cells involved in the inflammatory response are typically rich in ω-6 arachidonic acid, but the contents of arachidonic acid and of ω-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be altered through administration of EPA and DHA. Metabolites of arachidonic acid are mediators of inflammation. EPA also gives rise to eicosanoids and these may have different properties from those of arachidonic acid-derived eicosanoids. EPA and DHA give rise to resolvins which are anti-inflammatory and inflammation resolving. Thus, fatty acid exposure and fatty acid composition of human inflammatory cells influences their function [1]. In this study, the anti-inflammatory properties of fixed lipids extract (LE) from cod liver were studied *in vitro* with respect to fatty acid metabolism by COX and LOX enzymes and histamine influx. LE is an extract that contains natural lipids with 24% of ω– 3 (mainly EPA and DHA), 12% ω– 7 and 31% ω– 9 PUFAs. It was shown that LE is a potent inhibitor of COX-2 (IC_{50} = 9,8 μg/ml) and 5-LOX (IC_{50}= 16,2 μg/ml). The histamine release from the rat basophils cell line RBL1 induced by compound 48/80 was inhibited 2 fold by LE at the concentrations of 1 – 16 μg/ml. **Reference:** [1] Calder P. Eur J Pharm 2011; 668: S50-S58

PM11 Molecular cloning and functional characterization of an NADPH:cytochrome P450 reductase from a tropical medicinal plant *Scoparia dulcis*

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Cytochrome P450 monooxygenases (P450 s) are commonly involved in biosynthesis of endogenous compounds and catabolism of xenobiotics. All known plant P450 reactions depend on the associated activity of an NADPH:cytochrome P450 reductase (CPR) that catalyzes the transfer of electrons from NADPH via FAD and FMN to the prosthetic heme group of the P450 protein. We isolated a cDNA for CPR gene (designated as *SdCPR*) from *Scoparia dulcis* leaf. The *SdCPR* cDNA contains an open reading frame encoding a protein of 713 amino acids, with a predicted relative molecular weight of 78.5 kD. By aligning deduced sequence of SdCPR with other plant CPRs from taxonomically diverse species all functional domains involved in the binding of the P450, and cofactors of FMN, FAD and NADPH, were identified. Amino acid sequence comparison showed that the *Scoparia* CPR share high sequence identities with CPRs from other flowering plant species (66 – 80%). It belong to the Class I of dicotyledonous CPR. To characterize the activity of SdCPR, enzyme was produced in *Escherichia coli* as recombinant protein. After solubilization from membrane and affinity purification, the purity of the SdCPR protein was verified via SDS-polyacrylamide gel electrophoresis. Strong cytochrome c reductase activated were observed for SdCPR when NADPH was added. By contrast, NADH did not support either SdCPR for the reducing reactions, indicating that SdCPR utilize NADPH specifically as the electron donor. Compared to the electron donor (NADPH), the requirement of CPR for electron acceptor is relatively less specific: cytochrome c, $K_3Fe(CN)_6$, and dichlorophenolindophenol (DCPIP) can all serve as acceptor. Currently, we are evaluating the ability of SdCPR to support P450 activity using an Arabidopsis P450, CYP73A5. The expression of *SdCPR* in *Scoparia* plant was investigated by real-time PCR, which showed that transcripts of *SdCPR* were present in all tissues examined, including leaf, stem and root.

N. Miscellaneous

PN1 *Alpha-amylase Inhibition* and Antioxidant Activities of Methanol Extract and Fractions of *Raphia hookeri* (Palmae)

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Diabetes mellitus is caused by the abnormality of carbohydrates metabolism which is linked to low blood insulin level or insensitivity of target organ to insulin. The digestion of starch by α-glucosidases contributes to sharp increase in blood glucose resulting in postprandial hyperglycemia which has been implicated in the development of type 2 diabetes. The inhibition of these enzymes is a useful tool in the management of hyperglycemia and type 2 diabetes. In this study, methanol extract of *Raphia hookeri* stembark and its fractions were investigated for anti-diabetic ability using *in vitro* α-amylase inhibitory potential. The results showed that the extract prevented the digestion of carbohydrates by inhibiting α-amylase in a dose dependent manner with maximum inhibitory effect (72.86%, 1.00 $mgmL^{-1}$). The aqueous fraction demonstrated highest inhibiton (67.22%, 0.50 $mgmL^{-1}$) which was less than acarbose (68.90%, 0.08 $mgmL^{-1}$) used as positive control. The hexane, chloroform and ethylacetate fractions showed lower inhibitory activity (12.16, 9.06, 35.97%) respectively. The total phenolic content of the *P. osun* extract measured using Folin Ciocalteu reagent in terms of gallic acid equivalent (GAE) was found to be 109.10 ± 3.24 mgg^{-1}. The antioxidant activity of the extract increased with concentration and the radical scavenging activity of the aqueous fraction (93.66%, 0.10 $mgml^{-1}$) was comparable to ascorbic acid (0.1 $mgml^{-1}$) used as control. The hexane, chloroform and ethylacetate fractions however demonstrated lower radical scavenging activity (7.79, 8.20 and 68.30%) respectively. This study indicates that the extract of *Raphia hookeri* has α- amylase inhibitory and antioxidant activities which reside mainly in the aqueous fraction. **References:** [1] Kwon, Y. I., Vattem D. A., Shetty, K. (2006): Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. Asia Pacific J. Clin. Nutr. 15:107 – 118.

PN2 Anti-oxidant and anti-glycation constituents from *Ziziphus oxyphylla* and *Cedrela serrata*

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Ziziphus oxyphylla and *Cedrela serrata* are Pakistan flora species with a reported use in jaundice and against liver diseases[1], and with antioxidant and DNA protecting properties[2], respectively. In the present study a bioassay-guided fractionation procedure was used to isolate antioxidant constituents from the leaves of *Z. oxyphylla* and *C. serrata*. Ethyl acetate fractions showed the higher DPPH scavenging activity and therefore they were further subjected to purification by flash chromatography. Seven compounds (**1**-**7**) from *Z. oxyphylla* and five (**8**-**12**) from *C. serrata* were isolated through semi preparative HPLC and were evaluated in various radical scavenging assays. Compounds **1**, **2** (quercetin-3-*O*-glucoside and 3-*O*-galactoside in a different ratio), and **8** (kaempferol-3-*O*-glucoside), with IC_{50}= 5.33, 7.95 and 4.36 μg/mL, respectively, had significant DPPH scavenging activity compared to quercetin (3.6 μg/mL) (p= 0.05). Similarly, the superoxide and ABTS assays indicated IC_{50} values ranging from 0.21 to 0.91 mg/mL and 0.17 to 0.32 mg/mL, respectively. All isolated compounds were identified as flavonoids, more in particular quercetin and kaempferol glucosides (monoglucosides such as quercetin-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-rhamnoside, kaempferol-3-O-glucoside, kaempferol-3-O-galactoside, and diglucosides). Furthermore, these compounds were potent inhibitors of the formation of advanced glycation end products (AGEs) in a protein glycation assay. Most of the compounds had low IC_{50} values for AGEs inhibition, ranging from 0.53 to 0.84 mg/mL, compared to aminoguanidine, IC_{50}= 0.51 mg/mL, used as positive control. **References:** [1] Shah SR et al., 2006. Pak Journal of Weed Science Research. 12, 199 – 211. [2] Perveen F et al., M., 2012. Journal of Natural Products India 5, 207 – 213.

PN3 Anti-cholinesterase activity of 5-Theins recipes potentially used for Alzheimer's disease

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Background: In Thai traditional medicine, brain hypo-function, which is similar to Alzheimer's disease (AD), is due to declining in wind-element, blood circulation and neurological function. Review of Traditional Medicine scripture about wind-element, most frequently prescribed plants were group of 5-Theins as *Lepidium sativum* L.(*Ls*), *Nigella sativa* L.(*Ns*), *Cuminum cyminum* L. (*Cc*), *Foeniculum valgare* L.(*Fv*), *Anethum graveolens* L.(*Ag*). **Aims:** To study acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of the 5-Theins remedy and its ingredient. **Method:** 30 grams of each plant and the 5-Theins were separately extracted by 95% ethanol, evaporated and vacuum-dried at 45 °C. By Elman method, both enzymes activities were assessed. The constituents of each extract were determined by GC-MS. **Results:** All extracts at dose of 0.1 mg/ml exhibited higher BuChE inhibitory activity than that of AChE. The IC_{50} of BuChE inhibitory activity of *Ls*, 5-Theins(EtOH) and galantamine were 5.59,12.27 and 0.95 µg/ml. The constituents of each extract were shown in table.

sample	AChuEI activity	BuChuEI activity		Main constituents determined by GC-MS
	% inh. (0.1 mg/ml)	% inh. (0.1 mg/ml)	IC_{50} (µg/ml)	
Ns	26.16 (2.9)	21.41 (1.2)	> 200	eicosanoic acid, fatty acid
Ls	32.63 (2.9)	**91.45 (0.6)**	**5.59 (0.6)**	cumialdehyde, apiol, isoanethol, thymoquinone
Cc	27.64 (1.2)	41.35 (0.7)	*128.36 (5.3)*	cumialdehyde, eicosanoic acid
Fv	22.94 (0.6)	18.86 (1.2)	> 200	isoanethol, oleic acid ethyl ester, cumialdehyde
Ag	29.61(1.8)	18.94 (1.2)	> 200	diterpene derivative, L-carvone
5-Theins (EtOH)	29.5 (0.9)	**80.77 (1.0)**	**12.27 (0.7)**	oleic acid ethyl ester, linoleic acid, apiol, cumialdehyde
Galantamine	93.68 (1.0)	74.27 (0.9)	0.95 (0.03)	

Conclusion: The combined recipe had synergistic effect on BuChE inhibitory activity more than single herb except *Ls*. These results revealed the potential used of *Ls* and the 5-Theins for AD treatment. *"This work was supported by research fund of Thammasat University."*

PN4 Green extraction techniques with highly efficient non-conventional reactors: a screening of secondary metabolites by LC-MS and GC-MS

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The design of efficient and sustainable extraction methods for vegetal matrices has been a hot research topic over the last decade. Nowadays, the tendency is to perform extractions using non-conventional techniques such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). These eco-extraction techniques may lead to effective process intensification reducing the extraction time, improving selectivity and extraction yields. Conventional extraction processes are quite laborious, time- or energy-consuming, involve large amounts of solvents and may cause a high risk of degradation processes and the partial loss of volatiles. The efficiency of the proposed techniques will be shown by two independent studies that deal with different vegetal matrices and various analytical approaches. The aim of the first study was the design of a qualitative workflow strategy for screening the composition of marker fractions from little investigated crude extracts obtained from the aerial parts of six endangered alpine plant species (*Cicerbita alpina, Asparagus acutifolius, Chenopodium bonus henricus, Levisticum officinale, Silene vulgaris, Spirea aruncus*). The work was focused on a rapid MAE of phenolics by means of a design of experiments approach followed by a rational use of various analytical tools (UHPLC-PDA-TOF-MS and GC-MS) for the dereplication of crude extracts. For the first time in the six plants, a series of secondary metabolites were detected and/or unambiguously identified by selective algorithms of LC peak annotation. The second study compared classic extraction methods (maceration and hydrodistillation) with new highly efficient microwave and ultrasound reactors (batch and flow) for MAE and UAE of dry and fresh plants (*Mentha* sp., *Eugenia caryophyllus, Actinidia deliciosa*). The volatile fractions were characterized by HS-GC/MS. This work was carried out under the auspices of the Alcotra Eco-Extraction Transfrontalière project (France-Italy).

PN5 Hypoglycemic Effect of *Bromelia plumieri* (E. Morren) L.B. Sm., leaves in STZ-NA-Induced Diabetic Rats

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Diabetes mellitus is defined as hyperglycemia that is associated with inadequate insulin secretion, either in the presence or absence of impaired insulin action. Type 2 diabetes is characterized by tissue insulin resistance combined with a relative deficiency in insulin secretion. An individual may present primarily with either insulin resistance or beta cell deficiencies, and these abnormalities can range from mild to severe. This study confirms the hypoglycemic effects of two extracts obtained from the Bromelia plumieri (BP) plant in streptozotocin-nicotinamide induced diabetic rats (STZ-NA). BP has been traditionally used in the municipality of Hidalgo, Mexico, to treat type 2 diabetes. Two different BP extracts were prepared and tested. The first extract was a water extract (WE), similar to that traditionally used to make tea, and the second extract was an ethanol:water extract (EWE). The extracts (WE at 35 and 350 mg/kg, and EWE at 30 and 300 mg/kg) were tested to determine whether hypoglycemia occurred after administration of the extracts. Phytochemistry: Two different extracts were prepared, n-hexane and butanol, to determine the presence of alkaloids, terpenes and flavonoids. The Drug Extraction Ratio (DER) was 8:1 in the WE, 9:1 in the EWE and 164:1 BE. Using TLC, we confirmed that the main compounds found in the plant leaves are glycosylated flavonoids, which were present in all the extracts. The extracts that were administered to the NA-STZ induced diabetic rats produced a significant hypoglycemic effect as compared with the control group, similar to that achieved with glibenclamide. We also determined that flavonoids were the main components of BP leaves. The results presented here support the hypothesis that extracts obtained from this plant have hypoglycemic effects, which are in agreement with the traditional uses of this plant.

PN6 Alfa-glucosidase inhibiting activity of five Mexican plants used in the treatment of type 2 diabetes

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Diabetes mellitus is defined as hyperglycemia that is associated with inadequate insulin secretion, either in the presence or absence of impaired insulin action. Type 2 diabetes is characterized by tissue insulin resistance combined with a relative deficiency in insulin secretion. An individual may present primarily with either insulin resistance or beta cell deficiencies, and these abnormalities can range from mild to severe. Among glucose-lowering medications, α-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks. The root of *Smilax moranensis* M. Martens y Galeotti, Smilacaceae (SM), the stem of *Tournefortia hirsutissima* L., Boraginaceae (TH), the cortex of *Rhizophora mangle* L., Rhizophoraceae (RM), the cortex of *Cecropia obtusifolia* Bertol, Urticaceae (CO), and the leaves of B*romelia plumieri* (E. Morren.) L.B. Sm., Bromeliaceae (BP), are used in Mexican traditional medicine to treat type 2 diabetes; our work group proved the hypoglycemic effect of these plants. In the present study, we tested the extracts with respect to their α-glucosidase inhibitory activity, without excluding other possible mechanisms of action. Our results in nicotinamide-STZ diabetic rats loaded with maltose showed that all the plants extracts decreased plasma glucose significantly from 30 min compared with the diabetic control group. In vitro assays of rat intestinal α-glucosidase showed that CO decreased activity by 45% at 1000 µg/ml and BP by 30% at 2000 µg/ml, while SM, TH and RM showed less than 20% inhibition at 1000 µg/ml. These results suggest that inhibition of glucose absorption is not the main mechanism through which these plants reduce plasma glucose and contribute to understanding the mechanism of action of these plants on glucose metabolism

PN7 Performance of *Andrographis* (Kalmegh) in tree based cropping systems under dryland conditions

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A field experiment was conducted with andrographis in the existing plantations of amla and terminalia at College of Agriculture, Rajendranagar, Hyderabad during *kharif* seasons of 2008 and 2009 to study the feasibility of medicinal plants under shade. Shade-tolerant and rhizomatic MAPs can be grown on a long-term basis in widely spaced plantations ([1] Rao *et al.*, 2004). The soil of the experimental site was red sandy loam. The treatments consisted of three cropping systems as main plot treatments and six INM treatments as sub plot treatments. The treatments were laid out in split plot design and replicated thrice. The results revealed that growth parameters of andrographis *i.e.*, plant height, dry matter production and leaf area per plant were found maximum in sole cropping of andrographis followed by intercropping in amla and terminalia. Herbage yield (kg ha^{-1}) of andrographis and andrographolide content (%) were the highest in sole cropping of andrographis followed by intercropping in amla. Herbage yield (5395.8 and 5192.6 kg ha^{-1} in first and second year) of andrographis were significantly more with INM practice *i.e.*, M_5 (20 kg N ha^{-1} through urea + Vermicompost @ 2 t ha^{-1}). **References:** [1] Rao, M. R., Palada, M. C and Becker, B. N. 2004. Medicinal and aromatic plants in agroforestry systems. *Agroforestry systems*. 61: 107 – 122.

PN8 Understanding the mechanism of antidiabetic activity and efficacy of functional foods against advanced glycation end products: *Nigella sativa* and *Moringa oleifera*

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The current focus of diabetes research is moving away from the control of acute metabolic imbalances to the prevention of chronic complications such as retinopathy, neuropathy, nephropathy and micro-vascular complications. Hyperglycaemia is characterized by the large fluctuations in the blood glucose level and this can accelerate the glycation of various proteins and formation of advanced glycation end products (AGEs) in diabetes patients. The anti-hyperglycemic activity of *Nigella* seeds and *Moringa* leaves have been proved *in vivo* and it was therefore considered worthwhile to investigate the underlying mechanism. Analysis of the antidiabetic activity and their role in the prevention of diabetic complications was done based on their digestive enzyme inhibitory activity and their potential in preventing the formation of early and advanced glycation end products. The antioxidant activity of the plant extracts was determined based on their reducing power and radical scavenging properties. The inhibition of enzyme activity was observed for both the starch digesting enzymes which is important for the prevention postprandial hyperglycemia. The antiglycation activity of the plant extracts was analyzed based on the formation of Amadori product (Fructosamine) and advanced glycation end products (AGEs). *Nigella sativa* was reported to be comparatively more potent inhibitor of α-amylase and α-glucosidase. The amadori and AGEs inhibitory activity was found to be upto 80% in the presence of *Nigella* seeds extract. *Moringa* leaves showed about 70% inhibition and the inhibitory activity was persistent with the incubation time. This signifies the anti-hyperglycemic effect of the plant extract and suggest that, the consumption of a diet rich with functional foods could be helpful in the maintenance of blood glucose level and preventing the development of hyperglycemia associated complications.

PN9 Metabolite transformation profiles in the detoxification process by fungal solid-state fermentation of a Brazilian physic nut cake (*Jatropha curcas*)

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A large amount of seed cake is generated as a by-product during biodiesel production from physic nut (*Jatropha curcas*). This material has high protein and fiber content, which make them interesting for use as livestock feed. However the presence of toxic phorbol esters (PE) restricts its use [1]. In a previous study a Brazilian *Jatropha* seed cake was used as a cultivation medium for *Penicillium simplicissimum* in solid-state fermentation (SSF) to obtain lipase and for the simultaneous detoxification [2]. In this study in order to contribute to the understanding of the mechanism involved in this *Jatropha* seed cake detoxification process, the secondary metabolite transformations were analyzed by comparing the methanol extract profiles before and after the detoxification. The liquid-liquid partition of the methanol extracts using hexane and methanol/water 9:1 yielded fractions that were analyzed by thin layer chromatography (TLC), infrared spectroscopy (IR) and nuclear magnetic resonance techniques (^1H and ^{13}C NMR). The hydro-methanol fractions were acetylated and further analyzed by TLC, IR and NMR, while the hexane fractions were methylated and then analyzed by high performance liquid chromatography (HPLC) [3]. The results showed the complete metabolism of the carbohydrates present in the non-fermented cake as well as the hydrolysis products of the remaining oil. No traces of either by-products from the PE or new fungal metabolites were detected so far. **Acknowledgement:** CNPq – Brazil **References:** [1] *Li, C-Y, Devappa, RK; Liu, J-X, Lv, J-M, Makkar, HPS, Becker, K.* Food Chem. Toxicol. 2010; 48: 620 – 625. [2] *Godoy, MG.* Value addition and biodetoxification of castor bean cake *(Ricinus communis)* and physic nut cake (*Jatropha curcas*) by solid-state fermentation. PhD Thesis. Universidade Federal do Rio de Janeiro, 2013. [3] *Andrade, DF, Mazzei, JL, d'Avila, LA.* Rev. Virtual Quim. 2011; 3: 452 – 466. Available at www.uff.br/rvq

PN10 Effect of *Urtica dioica* hydro-alcoholic extract on serum testosterone level of STZ – diabetic mice

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Diabetes has been associated with reproductive impairment in both men and women. *Urtica dioica* has been used extensively in Iranian traditional medicine in the treatment of diabetes. The aim of our study was to assess the effect of hydro-alcoholic leaf extracts of *Urtica dioica* on serum testosterone level in diabetic mouse model. This study was carried out on 40 Swiss-albino mice were randomly divided into 3 groups: a control group (n = 10) received an equal volume of distillated water once daily by means of intraperitoneal injection, *an urtica dioica* group (n = 10) that received single daily injection of *Urtica dioica* extract *at* 100 mg/kg, and a diabetic group which received only Streptozotocin (STZ) (n = 20). The third group was divided into two identical ones including STZ group and treatment group in which were used to induct diabetes using STZ (50 mg/kg BW; Sigma USA) within 5 days. Blood glucose levels were checked randomly out in blood collected from the tail vein a week after the last injection. Moreover, the blood glucose levels of > 250 mg/dl was considered as diabetic mice. Then, in the treatment group received *urtica dioica* extract (100 mg/kg/day) for 4 weeks while the control group just received an equal volume of physiological serum (IP) daily. After 4 weeks, 1cc blood sample was collected from abdominal aorta and the serum testosterone levels were measured. The mean serum total testosterone in the groups was not statistically significantly different ($p > 0.05$).[1,2] **References:** [1] Atalay B, Ilhan F, Gulyuz F, Karaca M, Cihat A. Assessment of the Histopathological Changes Occurring in the Testis of the Mice Suffering from Experimental Diabetes Induced Using Alloxan.. Journal of Animal and Veterinary Advances. 2009,8(10): 1929 – 1935. [2] Khaki A, Nouri M, Fathiazad F, Ahmadi-Ashtiani HR, Rastgar H, Rezazadeh Sh. Protective Effects of Quercetin on Spermatogenesis in Streptozotocininduced Diabetic Rat. Journal of Medicinal Plants.2009, Volume 8: 57 – 64.

PN11 Hypolipidemic effect of hydro-alcoholic extract of *Urtica dioica* in STZ – diabetic mice

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Urtica dioica has been used extensively in Iranian traditional medicine in treatment of diabetes. The aim of our study was to assess the effect of hydro-alcoholic leaf extract of *Urtica dioica* on serum lipid level in diabetic mouse model. To perform the study the fresh leaves of *UD* were collected. This study was carried out on 40 Swiss Albino male mice, randomly distributed in 4 groups. All the animals were weighed prior to administration of streptozotocin (STZ) and after treatment, and the range of weight was between 30 – 40gr. Diabetes was induced by multiple intraperitoneal (IP) injection (50 mg/kg) of STZ in 20 mice (Diabetic groups). Hydro-alcoholic extract of *UD* was injected (IP) to one of the diabetic groups and one of our control groups with the dosage of 100 mg/kg for 28 days. Two other groups received water (IP) injection. After 4 weeks,1cc blood sample was collected from abdominal aorta and the serum lipid level was measured. Our studies showed significantly lower levels of cholesterol and Triglyceride in mice that were treated with leaf extract of *UD* as compared with the control group of mice ($p < 0.05$) but there was no significant correlation between LDL and HDL of treatment and control groups. The mean cholesterol level in treatment group which was diabetic and received *UD* extract for 4 weeks, was 62 mg/dL while the mean in STZ group which was diabetic and received an equal volume of water was 100 mg/dL. The results of this study suggest that leaf extract of *Urtica dioica* decreases cholesterol in STZ induced diabetic mice.[1,2] **References:** [1] Atalay B, Ilhan F, Gulyuz F, Karaca M, Cihat A. Assessment of the Histopathological Changes Occurring in the Testis of the Mice Suffering from Experimental Diabetes Induced Using Alloxan. Journal of Animal and Veterinary Advances.2009,8(10):1929 – 1935. [2] Khaki A, Nouri M, Fathiazad F, Ahmadi H, Rastgar H, Rezazadeh Sh. Protective Effects of Quercetin on Spermatogenesis in STZ induced Diabetic Rat. Journal of Medicinal Plants. 2009, Volume8:57 – 64.

PN12 Liposomes for dihydroartemisinin delivery to cancer cells: development, characterization and *in vitro* studies

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Dihydroartemisinin (DHA) is the most potent anticancer artemisinin-like compound and induce cancer cell death by apoptotic pathways; it is a poorly water soluble compound with low bioavailability and low half-life (34 – 90 min). Therefore, the development of the new formulation of DHA that enables quick availability to the body is of great need. Conventional and stealth liposomes prepared according to the film hydration method, were fully characterized by particle size, zeta potential, polydispersity index, drug entrapment efficiency and TEM analysis. Stability in presence of blood proteins like albumin was evaluated. Cellular uptake of liposomes by flow cytometry and fluorescence microscopy analysis was investigated. In addition *in vitro* cytotoxicity studies in the cell line MCF-7 were carried out by sulforhodamine B assay. Both formulations showed average size around 100 nm and good values of zeta potential (-30 mv) and encapsulation efficiency (70%). TEM analysis confirmed the presence of round-shape vesicles that remain stable and intact also in presence of albumin. The higher cytotoxicity of conventional liposomes (IC_{50}= 48.2 μM) compared with stealth liposomes (IC_{50}= 77 μM) was consistent with the notion that pegylated liposomes might not interact directly with the tumor cells. The two liposome formulations developed represent a successful attempt to incorporate dihydroartemisinin in liposomes with physical characteristics as drug carrier for parental administration.

PN13 Isolation, characterization and antimicrobial activity characterization of endophitic bacterial communities from *Echinacea* species: identification of bioactive molecules producing isolates from medicinal plants

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Endophytes are fundamental in plant life promoting their growth and producing bioactive molecules with important medical and biotechnological applications. The therapeutic properties of medicinal plants are therefore likely related also to their endophytic communities that can directly produce bioactive compounds and/or elicit plant metabolism and growth. We carried out the isolation and the molecular characterization of bacterial communities from the medicinal plants *Echinacea purpurea,* and *Echinacea angustifolia* with the aim to: i) study the variation of the endophytic communities from the soil to the internal tissues of the same species and among species, ii) functionally characterize the strains testing their resistance to antibiotics, heavy metals, hydrogen peroxide and gasoil, iii) identify bacterial isolates showing antimicrobial activities against human pathogens to establish a biobank specific for of such type of strains. Bacteria were isolated from leaves and roots and from the rhizosphere of the two medicinal plants; 16S rDNA sequencing and RAPD fingerprinting of 600 randomly selected isolated were carried out, followed by taxonomic identification. The functional characterization was performed testing bacterial growth on solid media supplemented with various substrates at different concentration. Finally the cross-streak method was used to test their antimicrobial activity *versus* ten strains of *Burkholdera cepacia* complex (Bcc), an opportunistic human pathogen causing infections in Cystic Fibrosis patients. Results highlighted a strong variation in the communities between the different plant species (although grown in physical proximity) and in different organs/soil in the same species, moreover a variability was observed in response to different growth conditions, suggesting a selection of the endophytic microflora by the plant species and tissue; finally, several isolates showed an antimicrobial activity against different Bcc strains.

PN14 Solid lipid nanoparticles for oral delivery of curcumin

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Despite multiple medicinal benefits, the therapeutical utility of curcumin is strongly limited by its poor aqueous solubility and low oral bioavailability. Our work was focused on the development and characterization of solid lipid nanoparticles (SLN) for the encapsulation of curcumin for oral administration. Ultrasound technique was employed to prepare Compritol® 888 ATO SLN and formulation parameters as sonication time and drug concentration were evaluated and optimised. The physicochemical characterization of curcumin-loaded SLN in terms of particle size, drug loading capacity, drug entrapment efficiency, TEM analysis and *in vitro* drug release, was carried out. Stability was investigated on storage conditions (4 °C) over one month. Parallel artificial membrane permeability assay (PAMPA) was also carried out to predict gastrointestinal absorption. The optimized formulation showed average size below 300 nm and good values of zeta potential (-33 mV) and encapsulation efficiency (80%). The presence of round shape SLN with homogeneous size distribution was confirmed by TEM analysis. There was no modification on the particle size neither on the zeta potential of the solid lipid nanoparticles, which maintained their original values over one month. Parallel artificial membrane permeability assay (PAMPA) showed a considerable increasing of the amount of curcumin permeated in the case of SLN suspension if compared with the saturated solution of curcumin used as control. We observe also a prolonged release profile that suggest that curcumin molecules are solubilized into the solid lipid matrix. Principal advantages of the developed SLN over the already reported nanocariers of curcumin are represented by the absence of usual solvents used for the preparation of the formulation and the use of very

safe tensides such as Plunorics. For these reasons our SLNs offer a promising delivery system for enhancing the oral absorption of poorly soluble drugs curcumin.

PN15 Bacterial endophytes from *Lavandula officinalis*: a possible source of medically relevant bioactive compounds producing isolates

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Endophytes play important roles in plant biology by promoting plant growth; they are also relevant in biotechnology as they produce bioactive molecules, such as novel antimicrobial compounds. Besides, it is likely that the therapeutic properties of medicinal plants are also influenced by their endophytic communities as bacterial strains can directly produce bioactive compounds and/or elicit plant metabolism to produce them. This study aimed at the isolation and molecular characterization of bacterial communities from the widely used aromatic and medicinal plants *Lavadula officinalis* and to check the inhibitory capacity of endophytic isolates toward human pathogens and build a collection of bacterial isolates showing antimicrobial activity. To this purpose bulks of 5 plants were built and cultivable bacteria were isolated from both surface sterilized tissues (roots stems, and leaves) and from the rhizosphere; sequencing of the 16S rRNA genes from 400 randomly selected isolated (100 isolates for each sample) was carried out, followed by taxonomic identification matching against RPD database. Antimicrobial activity testing was performed with the cross-streak method against Cystic Fibrosis opportunistic pathogens belonging to the *Burkholderia cepacia* complex (Bcc). Data obtained revealed that: i) a strong variation in the composition of the communities between the different plant tissue, ad example *Rhizobiales* constitutes the 45% of the endophytic community of the root, but are absent in the stem and leaves; the lowest diversity of species was registered in the stem whose communities is dominated by members of the *Pseudomondales*; ii) a low overall diversity in the composition was found; iii) many known PGPR species were identified; iv) several isolates exhibited a strong antimicrobial activity against Bcc bacteria. In conclusion our results suggested that medicinal plants appear to be an important source of interesting isolates with biotechnological applications potential.

PN16 Antimicrobial activity of essential oils toward clinical and environmental strains of the opportunistic pathogen of Cystic Fibrosis patients *Burkholderia cepacia* complex

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The rapid development of multidrug resistance pathogens is among the most serious threat to public health and to successful antibacterial treatment. The genus *Burkholderia* includes a variety of species, with opportunistic human pathogenic strains, with high resistance to many classes of antibiotics. A major role in resistance could be played by multidrug efflux pumps belonging to the RND superfamily. Essential oils, secondary plant metabolites, possess antibacterial, antifungal and antiviral properties and have been screened worldwide as potential sources of new antimicrobial compounds, alternatives to synthesis antibiotics to treat infectious diseases. Essential oils major components are derived from terpenes and terpenoids. These components seem to be able to damage bacterial cell membrane and to render it more permeable and to deplete the proton motive force. In our study we have screened by disk diffusion method, the antimicrobial activity of 6 different essential oils: clove (*Eugenia carophyliata*), lavender (*Lavandula hybrida*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), tea tree oil (*Malaleuca alternifolia*) and thyme (*Thymus vulgaris*) against 10 *Burkholderia* strains, both of clinical and environmental origin, and against 8 *B. cenocepacia* J2315 mutants impaired in RND transporters. Results demonstrated that oregano, thyme and clove essential oils exhibited considerable inhibitory effects against all the organisms under test even against ciprofloxacin resistant strains. Furthermore tests made on mutant strains demonstrated a high level of inhibitory activity by essential oils tested against the RND 4 mutant, in agreement with previous studies that have shown that this efflux system is a major cause of multidrug resistance in *Burkholderia*. The MIC determination for oregano, thyme and clove essential oils against each strain should be an obligatory step in the research of new bacterial inhibitors for the improved control of infectious diseases.

PN17 Schisandrae fructus extract showed antiproliferative effect on human prostate cancer cells

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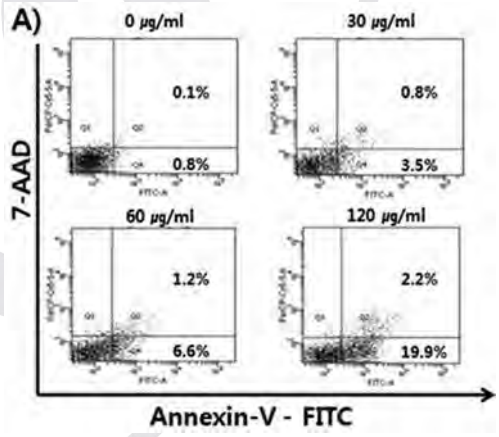
Schisandrae fructus (SF) from Schisandra chinensis (Turcz.) Baill. has traditionally been used to balance the level of body fluid and to strengthen kidney function. It has been reported that the SF extract has antioxidant, hepatoprotective, neuroprotective and anticancer effects. This study investigated an antiproliferative effect of SF extract on PC-3 human prostate cancer cells and analyzed active ingredients of SF extract qualitatively and quantitatively. We examined the antiproliferative effect of SF extract with MTT assay, DAPI staining and annexin-V/7-AAD double staining. The active ingredients of SF extract were identified by using HPTLC and HPLC/DAD. A SF-chloroform fraction inhibited growth of PC-3 cells and changed the morphology of nuclei in a dose dependent manner. A dose-dependent apoptotic cell death was also measured by flow cytometry analysis. The SF-chloroform fraction contained more schizandrin than other fractions. These results suggest that the SF extract and schizandrin may be a potential chemotherapeutic agent for the control of PC-3 human prostate cancer cells.



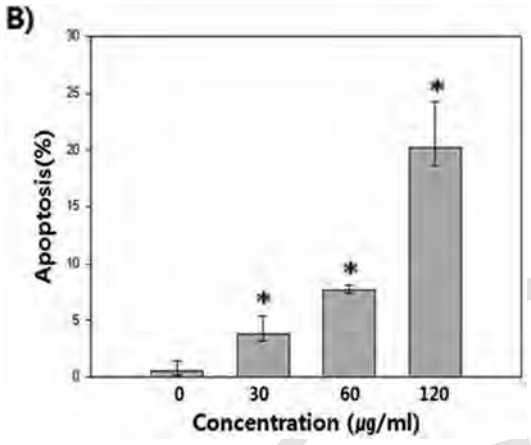


Fig. 1: Induction of apoptosis by SF-chloroform fraction in PC-3 cells. PC-3 cells were treated with SF-chloroform fraction (30, 60, 120 µg/ml) for 48 h. (A) Annexin-V/7-AAD double staining assay detected by flow cytometry. (B) The percentage of apoptotic cell for each treatment group. Values represent mean ± S.D. of three independent experiments (significant as compared to control, * $p < 0.05$).

PN18 Investigation of the potential use of a *Gloriosa superba* L. extract for the treatment of cancer

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Gloriosa superba L. (Liliaceae) is traditionally used in Ayurveda for several purposes, such as the treatment of gout, snake bites, intestinal worms, etc. *G. superba* contains colchicine, which has antimitotic properties and it has been used in the treatment of cancer. However, because of the low therapeutic index, it is not used as an anticancer drug anymore. *G. superba* contains not only colchicine but also other, very similar alkaloids. In this project the *G. superba* extract is evaluated for its potential use in the treatment of cancer. The hypothesis is that a combination of various active compounds in an extract may have more beneficial effects than the pure compounds, due to synergism and the presence of prodrugs such as glycosides. The 80% ethanolic extract of the *G. superba* seeds was phytochemically investigated and colchicine, 3-O-demethylcolchicine and colchicoside were isolated and identified by means of HPLC-SPE-NMR. In order to prepare a quantified extract with a known amount of colchicine derivatives for future *in vivo* experiments, an analytical HPLC-method was developed and validated. The 80% ethanolic extract contained 1.3% of colchicoside, 1.3% of 3-O-demethylcolchicine and 2.9% of colchicine. The cytotoxicity of the 80% ethanolic extract was also determined on three different human cancer cell lines and a high cytotoxic effect was observed on the tested cell lines with an IC_{50} value of 0.340 ± 0.015 µg/mL (MDA-MB-231 WT), 0.167 µg/mL (PANC-1) and 0.167 ± 0.008 µg/mL (HT-29) using the sulforhodamine B assay.

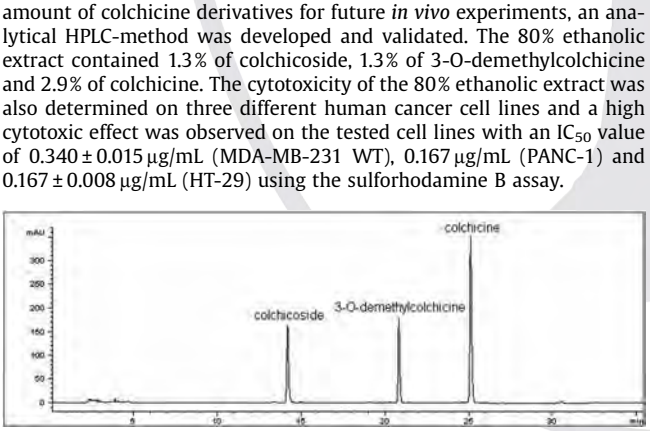

Fig. 1: HPLC chromatogram of the 80% ethanolic extract of the *G. superba* seeds.

PN19 Surfactant mediated extraction of phenolics compounds from *Syzygium aromaticum*, one of the richest source of natural antioxidants

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Surfactant mediated extraction is an innovative and green alternative for effective extraction and concentration of hydrophilic and lipophilic compounds from plants avoiding the use of organic solvents. Surfactants are classified according to the hydrophilic-lipophilic balance (HLB) which could influence the extraction of bioactive compounds. In the present work it was evaluated the influence of the HLB value, the type and concentration of surfactant, as well as the pH of the surfactant solution on the extraction of eugenol and phenols from clove (*Syzygium aromaticum*), a spice which has been classified as one of the richest source of plant phenols and antioxidants. Lecitin, Lauroyl polyoxyl-32 glycerides (Gelucire®) and others non ionic surfactants as Polysorbate 80, Sorbitan mostearate and Triton X-100 were tested. High Performance Liquid Chromatography (HPLC) and spectrometric results showed that higher HLB values improve eugenol and polyphenols extraction which was correlated with the antioxidant activity *in vitro*. Higher pH improved the extraction of eugenol which is explained by its pKa value. The extraction with a surfactant solution of HLB 15 at 5% was comparable with the concentration of eugenol and polyphenols achieved with the ethanolic (96% v/v) and hydroethanolic extraction (70% v/v) and significantly higher than the extraction with water. With regard to the surfactant type, Gelucire 50/13 and Geluciere 44/14, which are commonly employed in pharmaceutical formulations, presented good eugenol extraction. It can be concluded that water soluble compounds as polyphenols (gallic acid) and insoluble compounds as eugenol could be extracted from clove employing food and pharmaceutical grade surfactants facilitating the development of food and phytopharmaceutical preparations since organic solvents are not involved in the process.

PN20 Enzymatic activity and physiological status affect essential oils and polyphenolics production in *Artemisia alba* tissue cultures

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In previous research of*Artemisia alba* Turra, we reported that stimulation of root development*in vitro* was related to domination of monoterpenoids, while callus formation and rooting inhibition led to the prevalence of sesquiterpenoids in the essential oils of the aerial parts [1]. Interestingly, these two essential oil types correlated to the results of other authors concerning the oils of contrasting wild accessions of this species [2]. Here, we study the biochemical parameters of*A. alba* response to exogenous indole-3-butyric acid (IBA) and benzyl adenine (BA) treatment*in vitro*. The aerials of plants with a well developed root system (monoterpenoid domination in the oils in PGR-lacking control, as well as in IBA rooting stimulated media) were characterized by elevated CAT (EC 1.11.1.6), APX (EC 1.11.1.11) and GPOX (EC 1.11.1.7) levels, as compared with aerials of the plants from the "sesquiterpenoid group" (where combinations of IBA and BA led to suppressed rooting and callusogenesis). Interestingly, the plants from the "intermediate oil type" (bearing the terpenoid features of both root and callus forming plants) were characterized by a considerable drop of PAL (EC 4.3.1.24), CAT and GR (EC 1.8.1.7) and a drop of polyphenolics leading to marked elevation of lipid peroxidation and oxidative stress*in vitro*. In addition, electrophoretic profiles indicated differing enzymatic activities for the aerial, root and callus tissue samples. Modification of the essential oil profile through affecting morphological development *in vitro* will further be utilized for the targeted delivery of plant biomass with desired properties. Acknowledgments: This work was supported by the Swiss National Science Foundation in the framework of the Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZ0_142989; DO2 – 1153) **References:**
[1] Danova K et al. (2012) Natural Product Communications 7: 1 – 2. [2] Radulović N, Blagojević P. (2010) Natural Product Communications, 5, 1117 – 1122

PN21 Optimization of *in vitro* culture system for biomass and polyphenolics production in *Inula britannica* and *Sideritis scardica* Sofia 2 cultivar

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Inula britannica is used as an anti-inflammatory, anti-bacterial, anti-hepatitic, and anti-tumor agent [1]. Species of the *Sideritis* genus possess anti-inflammatory, antiulcerative, antimicrobial, vulnerary, antioxidant, antispasmodic, anticonvulsant, analgesic and carminative properties [2]. *Sideritis scardica* is a Balkan endemic species traditionally employed as expectorant for the treatment of pulmonary emphysema and angina pectoris [3]. *In vitro* culture was initiated by axillary buds formation of surface sterilized stem explants of *I. britannica*, collected from its wild habitat in Bulgaria, and of *S. scardica* – from sterile germinated seeds of the commercial cultivar Sofia 2, distributed on the Bulgarian market. Vitamin and plant growth regulators (PGR) were modified in order to optimize biomass production and polyphenolics accumulation *in vitro*. In *I. britannica* long-term growth of stock cultures was achieved in PGR-free medium. Biomass and polyphenolics formation were successfully stimulated by supplementation of Gamborg vitamins and a combination of N^6-Benzyladenine (BA) and Naphthylacetic acid. For Sofia 2 cultivar PGR-free medium resulted in a low multiplication index, has been unfavourable for long-term maintenance (over 4 weeks) and required more frequent subculture in order to avoid necrosis of explants. Addition of PGR allowed for optimization of biomass production with only slight reduction of the proportion of high molecular flavonoids as a part of total polyphenolic content. Acknowledgments: This work was supported by the Swiss National Science Foundation in the framework of the Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZ0_142989; DO2 – 1153) **References:** [1] Khan AL et al. (2010) Molecules 2010, 15, 1562 – 1577; [2] Gonzalez-Burgos E et al. (2011) J Ethnopharmacol 135: 209 – 225; [3] Ivancheva S, Stantcheva B (2000) J Ethnopharmacol 69, 165 – 172

PN22 Antioxidant potential of *Incarvillea emodi* (Royle ex Lindl.) Chatterjee

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Incarvillea Juss. is notable for being a temperate and herbaceous genus of the primarily tropical and woody family Bignoniaceae. It is composed of 16 species world wide and represented in Pakistan by one native species[1,2]. Most of the species occur in the Himalayas and S.W. China, where the diversity of the mainly herbaceous species appears to be related to the uplift of the Himalaya-Hengduan Mountains[1,2]. *Incarvillea* species are used for the treatment of hepatitis, diarrhea and infectious diseases. Some traditional herbal medicines of genus *Incarvillea* are used to treat rheumatism and relieve pain[1]. Biological activity studies on the genus have proved its antinociceptive, antiinflammatory, neurotrophic and antihepatic activities. Earlier phytochemical investigations on *Incarvillea* species resulted in the isolation of mainly alkaloids, ceramides, iridoids, flavonoids, and triterpenes[1]. In the present study, antioxidant potential of *Incarvillea emodi* collected from two different localities, Abbottabad and Kashmir in Pakistan, was evaluated against different free radicals. Aqueous extracts of the aerial parts and the roots of plant collected from Abbottabad and the aerial parts of plant collected from Kashmir were prepared, separately. Each extract was tested for its radical scavenging activity using DPPH, NO and SO radicals spectroscopically. The extracts showed concentration dependent radical scavenging ability on all the tested radicals. The most active extract, with 47.6168 µg/ml IC_{50} value, against DPPH radicals was found to be the extract obtained from aerial part of the plant from Abbottabad. The activity of the extract of plant from Kashmir was found to be weak. Isolation studies of the active compounds have been continuing. **Acknowledgment:** This study was supported by The Scientific and Technological Research Council of Turkey Program, No: 2216. **References:** [1] Fua, J. et al. Chemistry & Biodiversity (2009) 6. [2] Flora of Pakistan http://www.tropicos.org/

PN23 Antiplatelet and antithrombotic effects of *Morus alba* leaves extract

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Morus alba L. leaves has been used in fork medicine for the treatment of beriberi, edema, and diabetes and reported to have anti-obesity, anti-diabetic, anti-hypertension, liver protection, antiviral and antimicrobial activities. However, information on its anti-platelet and anti-thrombosis effects is limited. The current study was performed to examine the effects of M. alba leaves ethanol extract (MAE) in platelet aggregation and thrombosis. The anti-platelet activity of MAE was studied using rabbit platelets for in vitro determination of the extract effect on collagen-induced platelet aggregation, thromboxane B_2 (TXB_2) formation and serotonin secretion. The extract in vivo effects was also examined in arterio-venous shunt thrombus formation in rats. HPLC chromatographic analysis revealed that MAE contained rutin and isoquercitin. MAE significantly and dose dependently inhibited collagen-induced platelet aggregation with the 50 percent inhibitory concentration (IC50) of 0.42 mg/ml. MAE also attenuated serotonin secretion and thromboxane A_2 formation. In addition, the extract in vivo activity showed that MAE at 400, 200 and 100 mg/kg significantly and dose-dependently attenuated thrombus formation in rat arterio-venous shunt model by 52.3% ($p < 0.001$), 28.3% ($p < 0.01$) and 19.1% ($p < 0.05$), respectively.

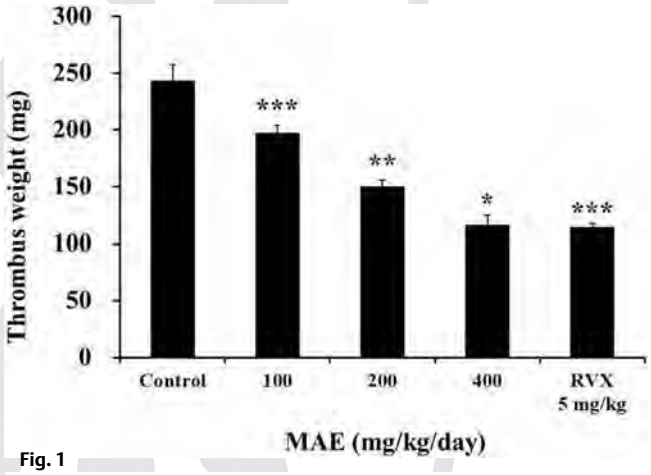

Fig. 1

PN24 The accumulation of free and bound phenolic acids in *Anethum graveolens* L. *in vitro* cultures cultivated on the Murashige and Skoog medium variants – preliminary results

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Phenolic acids, constitute a biologically attractive group of compounds showing among others antioxidant, immunostimulating and anticancer properties. These compounds occur in plants either in the free form or bound in glycoside and/or ester structures. Plant *in vitro* cultures can be a rich source of these compounds. Our earlier studies proved that *Anethum graveolens* L. (Apiaceae) callus cultures cultivated on Murashige and Skoog (MS) medium were an interesting source of free phenolic acids. The aim of the present studies was to analyze the contents of free and bound phenolic acids in biomass cultivated on the same MS medium variants. The callus cultures were cultivated on seven MS medium variants supplemented with plant growth regulators (PGRs): BAP (cytokinin) and NAA (auxin) in the concentration range from 0.1 to 3.0 mg/l. Methanolic extracts from biomass from *in vitro* cultures and fruits from the native plant after acid hydrolysis (2 M HCl, 2 x 2 h) were analyzed for phenolic acid contents by an HPLC method. In total, eleven phenolic acids and cinnamic acid were analyzed. All examined extracts contained six metabolites. The main metabolites in the biomass from *in vitro* cul-

tures were: salicylic acid (SA), p-hydroxybenzoic acid (p-HBA), ferulic acid (FA) and vanillic acid (VA). The analysis proved a significant effect of PGRs concentrations in the tested MS medium variants on the contents of the metabolites under study. The total content ranged from 77.83 to 120.40 mg%, the content of SA from 33.92 to 61.46 mg%, p-HBA from 22.33 to 42.55 mg%, FA from 0.23 to 11.76 mg%, VA from 4.87 to 9.16 mg%. The largest content of phenolic acids was observed on the MS medium variant containing 0.5 mg/l BAP and 2 mg/l NAA. Fruit extracts analyzed for comparison contained SA (71.76 mg%) and syringic acid (46.76 mg%) as the dominating compounds while the total content amounted to 154.14 mg%. The biomass from *in vitro* cultures of *A. graveolens* can be a potential source of SA and p-HBA.

PN25 The accumulation of pharmacologically active lignans: γ-schizandrin and deoxyschizandrin in agitating cultures of Chinese magnolia vine (*Schisandra chinensis* (Turcz.) Baill.) – preliminary results

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Schisandra chinensis (Turcz.) Baill. (Schisandraceae), is an East-Asian medicinal plant species which is known mostly of adaptogenic, hepatoprotective, antioxidant and anticancer actions. Biological activity of the plant is attributed mostly to dibenzocyclooctadiene lignans, called the schizandra lignans. Shoot-differentiating callus solid cultures of this species established in our laboratory were capable of accumulation of some lignans. The aim of the next step of the study was to investigate the accumulation of these compounds in agitating shoot cultures. In the extracts from biomass the presence of two lignans, schizandrin and gomisin A was confirmed earlier. The accumulation of two other lignans: γ-schizandrin and deoxyschizandrin has been studied. The agitating cultures were maintained on two variants of Murashige and Skoog (MS) medium, differing in concentrations of the plant growth regulators (BAP and NAA [mg/l]: 2 and 2, and 3 and 1, respectively) under constant artificial light (4 W/m^2), in Erlenmayer flasks on a rotary shaker at 140 rpm. Methanolic extracts of biomass and lyophilizates of media (collected after a 4-week growth cycles) were used to determine γ-schizandrin and deoxyschizandrin contents using an HPLC method. Lignans were accumulated mostly in biomass extracts. The obtained amounts of deoxyschizandrin equaling on the tested MS medium variants 92.37 and 81.23 mg%, respectively. γ-Schizandrin was accumulated in many-fold lower quantities 15.77 and 10.68 mg%, respectively. The obtained amounts of deoxyschizandrin were higher than in fruits (60.72 mg%) and leaves (41.01 mg%) of native plant. The established agitating cultures of *S. chinensis* can be proposed as a rich potential source of deoxyschizandrin.

PN26 The accumulation of free and bound phenolic acids in *Anethum graveolens* L. *in vitro* cultures cultivated on the Linsmaier and Skoog medium variants – preliminary results

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Phenolic acids compose a group of plant metabolites possessing very valuable therapeutic properties. These compounds exhibit among others antioxidant, immunostimulating, anti-inflammatory and anticancer activities. They occur in plants as free compounds or are bound in glycosides and/or esters. Plant *in vitro* cultures can be a rich source of these compounds. Our earlier studies demonstrated that *Anethum graveolens* L. (Apiaceae) callus cultures cultivated on Linsmaier and Skoog (LS) medium variants are an interesting source of some free phenolic acids. The present studies aimed to examine the accumulation of free and bound phenolic acids in cultures maintained on the same LS medium variants. Callus cultures were cultivated on three LS medium variants supplemented with plant growth regulators: BAP (cytokinin) and NAA (auxin), in the concentration range from 0.1 to 2.0 mg/l. The contents of eleven phenolic acids (cinnamic acid and benzoic acid derivatives and depsides), and cinnamic acid were determined in methanolic extracts from biomass and from fruits of the native plant after acid hydrolysis (2 M HCl, 2 x 2 h) using an HPLC method. All extracts under analysis contained six metabolites. p-Hydroxybenzoic acid (p-HBA) and salicylic acid (SA) were the dominating metabolites in callus extracts. The concentrations of individual compounds and the total contents in biomass cultured on different LS medium variants were similar and amounted to 22.93 – 25.80 mg/% (p-HBA), 11.04 – 11.57 mg% (SA), and 45.51 – 49.38 mg% (total content). SA (71.76 mg%) and syringic acid (46.76 mg%) were the main compounds in extracts from fruits analyzed for comparison. The total content of phenolic acids in fruit extract was 154.14 mg%. The obtained results indicate that biomass from *in vitro* cultures of *A. graveolens* can be a potential source of p-hydroxybenzoic acid.

PN28 Cytotoxic activity of Enterolobium contortisiliquum fruit

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Cancer is a public health problem worldwide. According to WHO, 20 million people in the world suffer from cancer, a figure projected to rise to 30 million within 20 years. Cancer causes 7.1 million deaths annually (12.6% of the global total) (WHO, 2004). *Enterolobium contortisiliquum* (Vell.) Morong (Leguminosae) is a tree belonging to the Mimosoideae subfamily and widely populated in Egypt. The phytochemical study of *Enterolobium contortisiliquum* fruit revealed the presence carbohydrate/or glycosides, triterpenes/or sterols, saponins, flavonoids and protein. The mucilage, lipoidal matter, saponin and protein fractions were isolated from *Enterolobium contortisiliquum* fruit and their chemical profile was identified using GLC, GC/MS and amino acid analyzer. Cytotoxic activity of the isolated extracts as well as the crude extract (70% alcohol) have been carried out *in vitro* on HepG-2, MCF-7, and A549 human cell lines according to Mosmann, (1983). The obtained cytoxicity screening proved that the tested extracts have various cytotoxic activities.

PN29 On the fatigue reducing effects of Japanese green tea (*Camellia sinensis* (L.) Kuntze) from Uji

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Green tea is consumed in Japan since more than a thousand years for its alleged ability to reduce mental fatigue and for its general health improving effects, which are mainly attributed to its prominent phenolic constituents such as epigallocatechin gallate. However, comprehensive parallel examinations of its psychological, physiological, and immunological effects in humans have not been conducted up to now. In this randomized cross-over human trial – conducted with green tea from the famous plantations of Uji in Kyoto – we monitored all these effects in a coordinated way. After 120 min of highly fatigue inducing computer work, subjects were randomized into two groups. Group 1 conducted the green tea trial on the first day and the water control on the second day. In group 2, the order was reversed. Otherwise, all procedures were carried out identically. Data were collected by means of blood sampling for natural killer (NK) cell activity, electrocardiography (ECG) with power spectrum analysis for heart rate variability – especially for both high frequency (HF) values and the average ratios between low and high frequencies (LF/HF) – as an indicator for the function of the autonomic nervous system, electroencephalography (EEG) for P300 event related potentials (ERP), and a questionnaire for assessing subjective psychological reactions. All measurements were executed three times: before and after the computer fatigue inducing task as well as 30 min after drinking either green tea or water. The heart rate variability results indicate that green tea induces dominance of the parasympathetic nervous system,

while the P300 data demonstrate an improvement in attentiveness. Furthermore, NK activity was also notably elevated and fatigue scores were improved by green tea intake. Thus, this study comprehensively demonstrates the fatigue reducing effect of Uji green tea in psychological, physiological, and immunological parameters.

PN30 Effect of methanolic extract of *Ruta graveolens* on *Enterococcus fecalis* growth

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Introduction & aim: *Enterococcus fecalis* is responsible for often 90% of enterococci infections. It can causes bacteremia, urinary system ulcer, biliary and endocarditis in adult and meningitis and septicemia in pediatrics. Regarding to our previous study about hydro-alcoholic and aqueous of Ruta on the growth of Enterococcus fecalis and no effect on it, our aim in this study has been effect of this herb on *enterococcus fecalis* growth. **Materials & Methods:** In this investigation we used standard *Enterococcus fecalis* PTCC-1237 which prepared from collection of bacteria and fungi, scientific and industrial research organization. Effect of hydro-alcoholic and aqueous extract of *Ruta graveolens* on growth of bacteria has been evaluated by disc diffusion and serial dilution method and compared with eight prevalent antibiotics. **Results:** In the first test, none of disks with methanolic extract in the range of 10 to 40 µg/µl show any non-growth hallo. In the second test disks with 40 µg of methanloic extract in comparison with antibiotic disks did not avoid from growth of bacteria. In third test for determination of MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration) in all of test tubes except case test tube turbidity were seen that show the growth of bacteria and ineffectiveness of various amount of extract. **Conclusion:** It seems that this ineffectiveness is because of low antibacterial substance against the bacteria in methanlic extract of the herb and probably is because of high resistant nature of Enterococcus fecalis to antibiotics. **Keywords:** *Ruta*, *Enterococcus fecalis*, *methanolic extract*

PN31 Effect of different plant density and arrangements on qualitative and quantitative performance (flower dry weight, seed dry weight, flower diameter, number of flower in plant, flavonoid content) of *Calendula officinalis* L.

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In order to determine the influence of plant density and arrangement on quantitative and qualitative properties of Calendula officinalis L., different planting distances were tested in the research fields of the Soha Jisa agro-industry corporation in 2011 – 2012. The experiments were done in factorial form in a randomized complete block design with three replicates. Different distances between rows were R_1 =30 cm and R_2= 40 cm and plant distances within the row were I_1 =10 cm, I_2 =20 cm, I_3 =30 cm. Cultivation also was done by two arrangements of the plants: P_1 =rectangular and P_2 =zigzag. Studied characteristics were the number of flowers per plant, the flower dry weight, diameter and flavonoid content and the seed dry weight. The results showed that the plant density and arrangement had significant effects at 1% ($p \leq 0.01$) and 5% ($p < 0.05$) level on all measured characteristics. The maximum number of flowers per plant and the maximum flower dry weight was obtained by cultivation in a distance of 30 cm between the rows, 20 cm within the row and zigzag arrangement ($R_1 \times I_2 \times P_2$). The maximum flower diameter, seed dry weight and flavonoid content was obtained by cultivation in a distance of 30 cm between the rows, 30 cm within the row and zigzag arrangement ($R_1 \times I_3 \times P_2$).

PN32 Studies concerning the molecular complexation of Lippia sidoides essential oil in β-cyclodextrin by conventional and ultrasound assisted method

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Lippia sidoides Cham. is an aromatic herb from Northeast of Brazil, popularly known as pepper-rosmarin. This plant contains an essential oil (EO) with high concentration of thymol and carvacrol; comparable of thyme oil. The presence of these and other bioactive compounds give this specie important antimicrobial, larvicidal, molluscicides, and antioxidant activities. When exposed to oxygen, light or heat, EOs can undergo irreversible alterations in their physicochemical properties. Microencapsulation can be applied to transform these substances into more stable materials (lower volatility and less susceptibility to oxidation) providing easy handling (solid form). Formation of inclusion complexes of EO in cyclodextrins has been reported as an efficient method to improve stability of their terpenoid compounds. There are several complexation methods, and the slurry method followed by water removal by filtration and evaporation or by other drying method (ex. freeze drying or spray drying) is one of the most commonly used. Since the slurry preparation and drying conditions are the key factor involved in the encapsulation efficiency, the aim of this work was to investigate the effects of ultrasound (ultrasound assisted complexation) in the efficiency of formation of inclusion complexes of *L. sidoides* EO in b-cyclodextrin (b-CD) compared with conventional homogenization technique. The ratio EO:b-CD studied were 1:10 and 2:10, and the solid content of the aqueous slurry set at 30%. The prepared slurries were spray dried at temperature of 160 °C, gas flow rate of 60 m^3/h, feed composition flow rate of 4 g/min. EO content, thymol content, product mean diameter, moisture content, and process yield were the responses evaluated. The results showed that the use of ultrasound improved at least 1.2 times the complexation efficiency compared to the conventional homogenization method. The ratio EO:b-CD of 1:10 showed better performance with regard to the total EO retention.

PN33 Plant latex proteases with potential influence on blood coagulation and fibrinolysis

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Proteases are widespread in latices of different plant families (e.g. Apocynaceae, Asteraceae, Caricacea, Convolvulaceae, Euphorbiaceae and Moraceae) [1]. Their function has not been completely clarified yet; involvement in wound healing after injury and protection from predators appear logical. Potential use of plant proteases are in the food industry (e.g. cheese production), washing industry or in the pharmaceutical industry. For the latter one possible application is the blood coagulation and fibrinolysis process. Some latex-containing plants are already under investigation in this aspect, like for example *Synadenium grantii* Hook (Euphorbiaceae), *Wrightia tinctoria* R.Br. (Apocynaceae), *Ficus carica* L. (Moraceae) and *Euphorbia hirta* L. (Euphorbiaceae) [2, 3]. We analysed the *in vitro* influence of the protease-containing latex of *Euphorbia mauritanica* L. (Euphorbiaceae) on some parts of the coagulation and fibrinolysis procedure, by using different methods like a fibrin plate assay, specific substrates or In-Gel-Digestion. It shows thombin-like activity, since it leads to an opacity in the plate assay and a moderate conversion of two thrombin-specific fluorogenic substrates. However it also shows plasmin-like activity, since it is able to dissolve small fibrin clots and coverts two plasmin-specific fluorogenic substrates. In-Gel-Digestion of fibrin and fibrinogen chains confirmed the activity, but the cleavage sites appear to be different. **References:** [1] Domsalla, A., Melzig, M.F. (2008) Planta medica 74(7): 699 – 711. [2] Flemmig, M., Melzig, M.F. (2012) J Pharm Pharmacol. 64(8): 1025 – 1039. [3] Patel, G.K., Kawale, A.A., Sharma A.K. (2012) Plant Physiol Biochem. 52: 104 – 111.

PN34 Protection and Sustainable cultivation of *Arnica montana* L. in Maramureş (Romania)

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The nature park Parcul natural Munţii Maramureşului located in the north of the Romanian Carpathians is covering mountain-meadows, forest and a highly variable mosaic of cultural land consisting of hayfields, pastures and other small pieces of extended used farmland. But this valuable landscape is threatened. Especially traditional farmed meadows exhibiting high biodiversity are endangered by reforestation and the lack of maintenance due to rural depopulation. In the course of that the rare plant *Arnica montana* appearing on nutrient-poor open land is at risk to disappear. Furthermore the pressure on the park (illegal wood harvest and hunting) is quite high due to the poor economical situation of the local people. To contribute to lower this exploitation and to preserve the structure as well as the biodiversity of this old-established cultural land the sustainable cultivation of the medicinal plant *Arnica montana* by local peasants was initiated. The product Arnicae flos will be manufactured by the farmers and directly traded to a German purchaser. The prior ambition is to protect the arnica-meadows and their biodiversity by sponsoring renaturation and maintenance activities respectively with a part of the sales revenue and by using natural populations as seed-reservoirs to increase the motivation to protect them. Furthermore improving the economical situation of the people helps to decrease the exploitation of protected areas. The park administration, as the central institution of the project will benefit by active collaboration with local people and will organize further nature-protection and educative activities. In addition the participation of a nature park assures the environmental compatibility of all realized actions. With this concept, other medicinal and aromatic plants could be cultivated in Maramureş and further transnational research projects can be realized regarding cultivation-methods, chemoecology as well as the economical impact.

PN35 *Haemanthus coccineus* extract and its main alkaloid narciclasine exert potent anti-inflammatory effects *in vitro* and *in vivo*

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Haemanthus coccineus (Amaryllidaceae) extracts have been used in traditional African medicine against febrile colds and asthma. Interestingly, the extracts' main alkaloid, narciclasine, was recently reported to induce apoptosis in different tumor cell lines. Beyond this anti-cancer action, we hypothesized that HCE and narciclasine could exhibit an anti-inflammatory potential. Dried bulbs of *H. coccineus* were extracted using 60% (w/w) ethanol. The ethanol was largely removed and the remaining solution was partitioned with ethyl acetate. The organic phase was separated and dried (DER 50:1). The resulting *H. coccineus* extract (HCE) contained 2.2% narciclasine. In a croton oil-induced ear edema model in mice, HCE was found to clearly reduce edema formation upon oral application (450 mg/kg). *In vitro*, HCE (3 ng/ml – 10 μg/ml) concentration-dependently inhibited the proliferation of lymphocytes and the synthesis of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) in macrophages. Moreover, HCE decreased the TNFα-induced adhesion of neutrophils to human endothelial cells (ECs) and the surface expression of EC adhesion molecules (ICAM-1, VCAM-1, E-selectin) without affecting EC viability. Also the main alkaloid narciclasine (1 nM – 10 μM) clearly reduced adhesion molecule expression without inducing cytotoxicity. Interested in the underlying mechanisms of action, we could reveal that HCE as well as narciclasine attenuated the TNFα-triggered NF-κB-dependent gene expression in a reporter gene assay. HCE treatment also reduced the DNA-binding activity of NF-κB (gel shift assay). However, surprisingly, HCE and narciclasine did not affect the phosphorylation and translocation of the NF-κB p65 subunit (Western blot and microscopical analysis). This interesting phenomenon is currently under further investigation. In conclusion, our study highlights *H. coccineus* extract and narciclasine as novel promising anti-inflammatory approaches.

PN36 Relationships between endophytic fungi and their hosts: impacts on drug quality of medicinal plants

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Endophytic fungi or endophytes widely present inside the healthy and living tissues of plants and are important components of plant microecosystems. Over the long period of evolution, some co-existing entophytes and their host plants have established a beneficial relationship, which can significantly influence the formation of metabolic products of plants, then impact on the crude drug quality of medicinal plants. This paper will mainly focus on the increasing knowledge of relationships between endophytic fungi and medicinal plants through reviewing of research results in the last 30 years. The endophytes can be considerably affected by factors, such as the genetic background, the age, and environmental conditions of their hosts. On the other hand, the endophytic fungi can also confer profound impacts on their host plants by enhancing plant growth, increasing fitness, strengthening tolerances to abiotic and biotic stresses, and promoting the accumulation of plant-secondary metabolites. All these are important for the production of bioactive components in their hosts such as medicinal plants. Hence, it is essential to understand the beneficial relationships of endophytic fungi and their host medicinal plants. Such knowledge can be well exploited and applied for obtaining better drugs from medicinal plants.

PN37 Growth arrest and Apoptosis of Dioscoreanone from *Dioscorea membranacea* against Human non-small cell lung cancer A549 cells

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Dioscoreanone (DN), 1, 4-Phenanthraquinone, has been previously isolated from the ethanolic extract of *Dioscorea membranacea* Pierre rhizome, and has shown to be potently cytotoxic to lung and breast cancer cells (1). This study further explored its cytotoxicity against two types of lung cancer cells including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), and also elucidated its molecular mechanisms. Cytotoxicity studies revealed that DN was specifically toxic to all three subtypes of NSCLC, but not to SCLC and normal lung fibroblast cells. The human lung adenocarcinoma cell line A549, one of the three NSCLC subtypes, was used to investigate the cytotoxic mechanisms of DN. The CFSE cell division assay showed that A549 cells treated with 15 μM and 30 μM DN proliferated more slowly than did control cells. Analysis of cell cycle distribution also indicated that both concentrations caused cell cycle arrest at the G2/M phase with the highest percentage of cells after 48 h incubation. Moreover, treatment of these cells with 30 μM DN for 72 h resulted in a 12 fold-increase in a sub-G1 peak corresponding to apoptotic cells as compared with control cells. With Annexin V/PI assay, phosphatidylserine translocation to the cell surface required for the early apoptotic cells was detected within 24 h, and progressively increased in a dose- and time-dependent manner. Importantly, such apoptosis was activated via caspase activation cascade because the highest activity of caspase 3 was found in A549 cells after treatment with 30 μM DN for 24 h. Moreover, the general caspase inhibitor Z-VAD-FMK completely suppressed DN-induced apoptosis in these cells, thus ascertaining caspase-dependent apoptosis as a major mode of cell death. This study disclosed the molecular mechanisms of DN for the first time and suggests that it may be a potential, natural apoptosis-inducing agent for NSCLC. **References:** [1] Itharat, A. et al. (2003) Org. Lett. 5, 2879 – 2882

PN38

In-vitro hepatoprotection study, free radical scavenging activity and gc/ms analysis of *Cyperus esculentus* essential oils from hydrodistillation and mae techniques

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A sample of essential oil is prepared from *Cyperus esculentus* tubers by hydrodistillation (HD) and another is prepared using microwave assisted extraction (MAE) techniques. When compared using GC/MS, they reveal the presence of at least 60 terpenoids and 7 non-terpenoid volatile chemicals. Sesquiterpenoids are the predominant constituents of both samples making 67.46% of the sample obtained by HD and 54.97% of that obtained by MAE. The percentages of monoterpenes in the same samples are 6.87% and 15.32% respectively. Free radical scavenging activity test (0.2% DPPH) is carried out to investigate the essential oils of *C. esculentus on a TLC* plate. A compound that appears as a yellow spot on the TLC plate due to the decolourization of the DPPH radical is identified as a sesquiterpene (2-naphthalenecarboxylic acid, 8-ethenyl-3,4,4a,5,6,7,8,8a-octahydro-5-methylene) using GC/MS. *The* essential oil obtained by MAE does not show IC_{50} effect on monolayer culture of rat hepatocytes up to a concentration of 1000 µg/mL. But, the essential oil obtained by hydrodistillation shows toxic activity with an IC_{50} value equal to 125 µg/mL. Using silymarin as a positive control, the essential oils obtained using both techniques show protective activity against paracetamol induced hepatotoxicity at a concentration of 12.5 µg/mL. Recently, it was concluded that different extraction techniques show diverse results in both the constituents and biological activities of plant materials (Sasidharan S, Chen Y, Saravanan D, Sundramand KM, Yoga Latha L (2011). Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *Afr J Tradit Complement Altern Med.; 8(1): 1 – 10*) From our study, the essential oils obtained using HD and MAE techniques differ in both the constituents and the tested biological activity.

PN39

Living on a volcano: medicinal plant uses of the Nabuclod Aeta, Mount Pinatubo, Philippines

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In the Philippines medicinal plants are becoming increasingly relevant in healthcare. This pilot study is the first documentation of traditional medicinal plant knowledge among the Nabuclod Aeta, an indigenous group of the Philippines directly affected by the Mount Pinatubo eruption. Through a combination of historical research and fieldwork, our aim is to explore medicinal plant uses among the Nabuclod Aeta and compare community plant uses with uses from historical sources dating from before the 1991 Mount Pinatubo eruption, to assess possible loss of medicinal plant knowledge. An ethnobotanical database was created using two historical sources pertaining to the Pinatubo Aeta (1) (2), and a shortlist of 21 plant species with medicinal uses reported by both sources was derived. Semi-quantitative interviews were conducted among 25 Nabuclod participants, including a questionnaire of the 21 species. Data analysis was conducted to determine frequency of plant uses between age groups, and the extent to which uses for these 21 species overlapped between historical sources and participants. 384 uses were reported within 24 illness-categories for the 21 species. The youngest age group's uses consisted mainly of popular plant knowledge nonexclusive to the Aeta, while the oldest age group exhibited a specialized knowledge of the traditional pharmacopoeia. Qualitative data supported and contextualized these findings. Despite the specialized plant knowledge still known among older Nabuclod Aeta, there is evidence that this has not passed on to the next generation. This community is at high risk of losing traditional medicinal plant knowledge in the near future. Further research is needed to preserve and promote this aspect of cultural heritage for improved community healthcare. **References:** [1] Fox, RB. (1952) Philipp. J. Sci. 81:173 – 414. [2] Pardo de Tavera, TH. (1901) *The Medicinal Plants of the Philippines*. (Thomas, JR Jr, transl.) Blakiston's Son & Co. Philadelphia.

PN40

Betulinic acid modulates cellular metabolism in an AMP-activated kinase-dependent manner

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Betulinic acid (BA), a pentacyclic triterpenoid abundantly found in nature, exerts a vast variety of bioactivities including anti-fungal, anti-bacterial, anti-inflammatory and anti-proliferative properties, both *in vitro* and *in vivo*. Recent evidence suggests that an alteration in the cellular phenotype, be it by physiological stimuli or pharmacologic intervention, is often inseparably linked with metabolic adaptations that secure sufficient supply with energy or building blocks needed for the respective cellular response. In this study we examined the metabolic changes induced by BA administration, with a main focus on cellular glucose metabolism. Using quiescent mouse embryonic fibroblasts (MEFs) as non-malignant and non-specialized cell model, we observed that BA (5 – 20 µM) dose dependently elevates cellular glucose uptake, an effect which is blunted in AMP-activated kinase (AMPK) knockout (ko) MEF. Exposure to BA consistently led to a transient activation of AMPK (1 – 3 hrs) in wildtype cells, as evident by increased phosphorylation at Thr172, and subsequently to an augmented mitochondrial mass after 24 to 48 hrs in an AMPK-dependent manner. These findings are mirrored by an increase in glycolysis and a transient drop (1 – 3 hrs) followed by a sustained rise (24 – 48hrs) in ATP production by oxidative phosphorylation after exposure to BA. These findings clearly demonstrate a metabolic flavour of BA administration which has also been indicated in reports on the anti-hyperlipidemic activity of BA and which may underlie or contribute to the pleiotropic actions of BA.

PN41

Inhibition of human cytomegalovirus IE gene expression by the extract of *Glycyrrhiza uralensis*

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The human cytomegalovirus (CMV) preferentially infects tumor tissues, and the accumulated CMV immediate early (IE) antigen may lead to tumor promotion and progression. The development of strategies to inhibit the expression and/or function of human IE antigen is an important goal to prevent and treat certain forms of cancer associated with human CMV. Recent pathological investigations demonstrated that HCMV can be found in Epstein-Barr virus-negative Hodgkin's disease, colorectal cancer, malignant glioma and prostate cancer cells. HCMV infection fails to transform susceptible normal human cells, but it modulates the malignant properties of cancer cells through its ability to interfere with a variety of cellular signal transduction processes. In the present study the $CHCl_3$ extract of *Glycyrrhiza uralensis* roots (chinese licorice) was studied on the expression of HCMV IE antigen expression in A549 (human lung adenocarcinoma) cells by immunofluorescence analysis. *G. uralensis* has been used since ancient times, and is one of the most popular medicinal plant in the world. Licorice contains several classes of secondary metabolites; with these compounds numerous human health benefits have been associated. In the present study the early phase of human CMV replication was used as a model of the early steps of malignant transformation in a modified in vitro system for measuring the antipromotion effects. Before this, the ID_{50} value of the extract was determined (49 µg/mL) on A549 cell line. The inhibition of IE antigen expression of CMV-infected cells in the presence of non-toxic doses of the $CHCl_3$ extract was evaluated. DMSO was used as positive control. It was observed that the extract caused a dose-dependent inhibition of IE antigen expression (growth inhibition dose ID_1= 76.3% and ID_{10}= 11% of control). This study was supported by the European Union and co-funded by the European Social Fund (TÁMOP-4.2.2.A-11/1/KONV-2012 – 0035), and Szeged Foundation for Cancer Research.

PN42

Screening of Hungarian mushrooms for xanthine-oxidase inhibitory activity

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Mushrooms represent a remarkable and yet largely untapped source of new, biologically active natural products. These compounds exhibit a

wide range of pharmacological properties (anti-inflammatory, anticancer, antioxidant and immunmodulatory activity) and possess a protective role against various diseases. Recently, it has become evident that some natural products have the ability to reduce oxidative stress by indirect antioxidant action. Xanthine-oxidase is a late enzyme of purine catabolism, source of reactive oxygen species generation in the pathogenesis of several diseases. More than 3000 mushroom species are native to Hungary, and only a small portion of these have been evaluated in terms of their potential pharmacological benefits. Aqueous and organic extracts of selected mushroom species from different families were screened for their xanthine-oxidase inhibitory activity. The fresh fruiting bodies were freeze-dried, and then were extracted with methanol. The methanol extracts were subjected to solvent-solvent partition, affording *n*-hexane, chloroform and the residual extracts. After extraction with methanol, the residual mushroom materials were dried and extracted with boiling water. Totally, more than 200 *n*-hexane, chloroform, 50% methanol or water extracts of different mushrooms were investigated. Some species exerted high xanthine-oxidase inhibitory activity, e.g. *Fistulina hepatica, Hypholoma fasciculare, Infundibulicybe geotropa*, *Tricholoma populinum*; others proved to possess moderate or weak activity. Further mycochemical studies are needed to identify the compounds responsible for the observed biological activity. **Acknowledgements:** this work was supported by the New Hungary Development Plan Projects TÁMOP-4.2.2/B-10/1 – 2010 – 0012 and TÁMOP - 4.2.2.A-11/1/KONV-2012 – 0035.

PN43 Assessment of the $Na_v1.2$ sodium channel activity of *Aconitum* diterpene and norditerpene alkaloids

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Voltage-gated sodium channels in sensory neurons have been found to play a crucial role in several chronic painful neuropathies of different aethiology. Pharmacons that exhibit a use-dependent blockade of these channels, like anticonvulsants, antiarrhythmics and local anesthetics, have been proven to be useful drugs in the treatment of chronic neuropathic or inflammatory pain states, epilepsy, migraine and neurodegeneration related to ischaemia. *Aconitum* species are traditionally used as painkillers, anaesthetics, cardiotonics and antirheumatic agents; characteristic compounds of these plants are the diterpenoid alkaloids. Analgesic effect of diterpene alkaloids is well-documented, and antinociception is a key component of this activity, which can develop by action on voltage-gated Na^+ channels. In the present study twenty-four C_{18}, C_{19} and C_{20} diterpene alkaloids, representing the structural diversity of *Aconitum* alkaloids, were evaluated for $Na_v1.2$ channel inhibitory activity by the whole-cell patch clamp technique. Pyroaconitine, ajacine, septentriodine, and delectinine demonstrated significant $Na_v1.2$ channel inhibition (42.4 – 57.0%) at 10 mM concentration; several other compounds (acovulparine, acotoxicine, hetisinone, 14-benzoylaconine-8-*O*-palmitate, aconitine, and lycoctonine) exerted moderate activity (21.9 – 30.3%), while the rest of the tested alkaloids were considered to be inactive. This is the first paper to report promising experimental results obtained in relation to a specific Na_v1 channel subtype inhibitory activity of diterpene alkaloids. On the basis of these results and by exhaustive comparison of data of previously published QSAR studies on diterpene alkaloids, certain conclusions on the structure-activity relationships of *Aconitum* alkaloids concerning $Na_v1.2$ channel inhibitory activity is proposed. **Acknowledgements:** This study was supported by the European Union and co-funded by the European Social Fund (TÁMOP-4.2.2.A-11/1/KONV-2012 – 0035).

PN44 Interactions of herbal constituents influences the effects of STW 5 on inflammatory processes and disturbed motility

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The herbal drug STW 5 (Iberogast®) contains nine individual plant extracts which affect inflammatory processes and disturbed motility in the gut. The aim of this study was to investigate the effect of individual constituents as well as selected combinations on different parameters related to inflammatory processes. STW 5, STW 5-II (a combination containing only 6 of the 9 extracts) and lemon balm reduced LPS (10 ng/ml)-induced lactate dehydrogenase (LDH) release from CaCo-2 cells by 74 – 75%. *Iberis amara*, peppermint, chamomile, angelica and milk thistle inhibited this parameter by 25 – 37%. Liquorice, caraway and celandine had no effect. The combinations of *Iberis amara* and peppermint as well as peppermint and milk thistle revealed synergistic or additive effects, whereas the combination of chamomile and angelica evoked additive or antagonistic effects depending on their compositions (i.e. relative ratios of individual extracts). STW 5, STW 5-II, *Iberis amara*, peppermint, liquorice, caraway, milk thistle and lemon balm inhibited LPS (100 ng/ml)-induced TNFa release from THP-1 cells to 51 – 67%. Chamomile and angelica revealed a more potent effect. The combinations of *Iberis amara* and peppermint as well as chamomile and liquorice had additive or antagonistic effects depending on their compositions. STW 5 and STW 5-II reduced the acetylcholine (ACh)-induced contractions in rat ileum preparations to 81 – 83%. The individual components inhibited the contractions to 83 – 91% excepting lemon balm which had no effect. The combinations of *Iberis amara* and peppermint as well as liquorice and caraway had additive or antagonistic effects depending on their compositions. Taken together our results allow greater insight into the interactions between individual herbal constituents of STW 5 and confirm the concept of multi-target actions.

PN45 Flaxseed oil affects insulin sensitivity in streptozotocine induced diabetic rats

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Introduction: The cell functions involved in the action of insulin receptor binding enzyme and transporter activities are controlled by membrane properties, and the amount of dietary fat as well as the nature of fatty acids regulates various steps in the biosynthesis of membrane phospholipids. Objective: To investigate the effect of flaxseed oil on improving erythrocyte membrane components and insulin sensitivity in diabetic rats. **Methods:** Thirty two adult male albino rats were used in this study and classified into four groups control, flaxseed oil, diabetic and treated groups. Fasting blood glucose and plasma insulin were estimated. Total lipids in the red blood cells membrane were extracted with chloroform/methanol method. Erythrocyte membrane total lipids, total cholesterol and triglycerides were determined. Fatty acids and phospholipids fractions were measured by HPLC. **Results:** flaxseed oil administration significantly increased the mean value of membrane α- Linolenic acid in flaxseed oil treated group compared to the diabetic group while it significantly decreased the mean value levels of Arachidonic acid, Linoleic acid and Oleic acid. In addition, the mean value levels of membrane cholesterol, triglycerides and phospholipids were significantly decreased in treated group compared to diabetic group indicating the role of flaxseed oil in improving the membrane components. The most important result in this study is the reduction of both insulin resistance and fasting blood glucose levels in the treated group compared to diabetic one. Conclusion: Flaxseed oil has an important role in enhancing insulin sensitivity and decreasing blood glucose in diabetic rats which may be due to the reduction of membrane cholesterol, triglycerides and phospholipids.

PN46 Phytochemical and Biological Investigation of *Carthamus tenuis* Boiss. growing in Egypt

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A phytochemical study of the aerial parts of *Carthamus tenuis* Boiss. led to the isolation and identification of six compounds. Caffeic acid 9-O-glucoside (**1**), quercetin (**2**), luteolin (**3**), 3'-methoxy luteolin (**4**), proline (**5**) and choline (**6**). They were isolated using several chromatographic techniques and identified with spectroscopic analyses (UV, IR, NMR and MS) in addition to comparison with reported data. The Immunosuppressive property of compound (**6**) (5 mg/kg b.wt.) was comparable to those of prednisolone (10 mg/kg b.wt.); while, compounds (**1**) and (**2**) (20 mg/kg b.wt., each) significantly decreased anti-SRBC titer in comparison to control group. Total phenolic content of methanolic and aqueous extracts of the plant was determined by Folin-Ciocalteu reagent and expressed as mg gallic acid equivalent per gram dry plant material (mg GAE/g dry wt.). Antioxidant activity was measured by improved ABTS method, using Trolox as a standard, total antioxidant activity was expressed in terms of Trolox equivalent antioxidant capacity (TEAC per g dry weight of plant). The phenolic content was 65.8 mg GAE/g dry wt. and 18.2 mg GAE/g dry wt. in methanolic and aqueous extracts, respectively. While the antioxidant activity was 163.9 TEAC/g dry weight and 29.8 TEAC/g dry weight in methanolic and aqueous extracts, respectively.

PN47 Anti-inflammatory, anti-bacterial and antioxidant activities of Thai medicinal plants for diarrheal treatment

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A Thai traditional remedy called Thatbunjob (TBJ) is normally used to treat abdominal pain, diarrheal disease and health promotion in the list of National herbal medicinal products A.D.2011 of Thailand. Its formula consists of 23 plants. The objective of this investigation was to study on antioxidant, anti-bacterial and anti-inflammatory activities of extract from TBJ and each plant component. The antioxidant assay by DPPH radical scavenging assay [1], antimicrobial assay by disc diffusion [2] and anti-inflammatory assay by inhibition nitric oxide release on lipopolysaccharide (LPS) induction in RAW 264.7 cells [3] were determined. The extraction of TBJ was imitated by traditional using (ethanolic and water extract gave drug: extract ratio as 100:9.85 and 100: 10.40). The results were found that water and ethanolic extracts of TBJ showed stronger antioxidant activity than BHT (EC_{50}= 7.22 ± 0.72, 13.52 ± 0.99 and 14.55 ± 0.69 μg/ml. respectively). The water extract of *Zingiber officinale* Roscoe as one component of TBJ exhibited the higher inhibitory effect on nitric oxide production in RAW 264.7 than indomethacin (IC_{50} = 2.06 ± 0.09 and 20.32 μg/ml). The ethanolic extract of TBJ also exhibited moderate inhibition of NO production (IC_{50}= 27.48 ± 1.25 μg/ml). The ethanolic extract of TBJ exhibited against six diarrheal strains such as *Vibrio. parahaemolyticus*, *Staphylococcusaureus*, *Vibrio cholerae, Shigella dysenteriae, Escherichia coli, Salmonella typhi* (MIC = 0.625,1.25,2.5,2.5, 5,5 mg/ml) and gentamycin as positive control. It was considered as moderate antibacterial activity. It concluded that TBJ extract had a potential therapeutic agent for abdominal pain, diarrheal disease and health promotion. **References:** [1] Yamazaki, K. et al. (1994) *Chem. Pharm. Bull*., 42: 1663 – 1665. [2] Tewtrakul, S. and Subhadhirasakul, S. 2008. *J.ethnopharmacol*: 120; 81 – 84.

PN48 Effect of three grocery herbs on growth of *Klebsiella pneumoniae in vitro*

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Introduction: Regard to using medicinal plants in medical therapy, it has specific role in treatment of diseases. Bacterial resistance to chemical antibiotics, have been elevated irregular consumption of herbs in the society. Groceries are the closer source for provision of herbal medicine in our society. In this study we evaluated the effect of three grocery herbs: *Chrysanthemum parthenium*, *Astragalus hamosus* and *Teucrium polium* on the growth of *Klebsiella pneumoniae* in vitro based on their antibacterial effect in traditional medicine. **Material & Methods:** In this study we used reference bacteria which prepared from microbiology laboratory of Guilan university of medical Sciences. Effect of hydro-alcoholic extract of all three herbs on growth of bacteria has been evaluated by minimal inhibitory concentration (MIC) and serial dilution method and compared with control tubes. **Results:** Extract in range of 10 to 400 μg/μl of all three herbs didn't avoid from growth of bacteria in MIC (Minimal Inhibitory Concentration). Concentration 200 μg/μl of *Astragalus hamosus* has an intermediate suspicious effect but it was not significant. **Conclusion:** Although herbs are suitable drugs for prevention and treatment of diseases in traditional medicine but there is no surveillance on the dispensation of herbs in groceries. Comparison of effect of natural confirmed herbs and grocery herbs in the same name is suggested. **Keywords:** grocery, *Klebsiella pneumoniae, hydro-alcoholic extract*

PN49 From inflammation to depression – St Johns wort as a therapeutic approach

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An increasing number of clinical and preclinical data shows that inflammatory processes may be involved in the etiology of depression [1, 2]. At least in some depressed patients inflammation markers are enhanced. An IFN-α-induced rise of TNF-α and IL-6 may induce depressive symptoms [3]. Psychic stress is not only a trigger of depressive symptoms, but also of a lower antioxidative capacity, similar to that in inflammatory diseases. As many herbal extracts have anti-inflammatory actions, the question is, whether these are also involved into the antidepressive action of St Johns wort [4]. It was addressed by a systematic data base search. St Johns wort extracts and their components have anti-inflammatory and anti-oxidative actions *in vitro* [5,6], *in vivo* [2,7] and, in dermatology, also clinically [8]. In a model of a stress induced depression, St Johns wort also normalized the lowered antioxidative capacity [2] and influenced gene expression of pro-inflammatory cytokines [7]. As St Johns wort extracts have anti-inflammatory properties, it is plausible that these are involved also in the therapeutic use in depression, and have possibly been underestimated up to now. **References:** [1] Dantzer R et al. 2008, Nature Reviews Neuroscience 9, 46 [2] Grundmann O et al. 2010, Neuropharmacology 58, 767 [3] Raison CL and Miller AH, 2011, Curr Psychiatry Rep 13, 467 [4] HMPC 2009, EMA/HMPC/101304/2008 [5] Birt DF et al. 2009, Pharm Biol 47, 774 [6] Kraus B et al. 2010, Planta Med. 76, 1340 [7] Jungke et al. 2011, Psychopharmacology 213, 757 [8] Schempp CM et al. 2002, Hautarzt 53, 93

PN50 Insecticidal Activity and Alkaloid Components of *Stemona* spp. in Thailand

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Stemona species have been traditionally used as natural pesticides and medicinal plants. Despite their diversity, the same vernacular name of "Non Tai Yak" has been used for various species in Thailand because of their similar root shapes. However, a variation in phytochemical consti-

tuents of different species was observed, leading to their different biological activities. This study determined the insecticidal activities of various species growing in Thailand. The samples representing 7 species and one unidentified species, probably closely related to *S. collinsiae*, were tested using chronic feeding bioassays with neonate larvae of the polyphagous pest insect *Spodoptera littoralis* Boisduval (Lepidoptera, Noctuidae). Potent insect toxicity was observed in all *S. collinsiae*, the unidentified species, and some samples of *S. curtisii*. *S. kerrii* as well as *S. aphylla* and *S. ruprestris* showed low to moderate activity. *S. tuberosa* and *S. phyllantha* showed no marked insecticidal activity. Stemofoline and didehydrostemofoline were found to be the major compounds in the active samples, clearly demonstrating the higher insecticidal effect than the other alkaloids. Thus, stemofoline and didehydrostemofoline sould be used as bioactive chemical markers for the quality assessment of *Stemona* roots, their extracts and final products for further pharmaceutical and agricultural developments.

PN51 Herbal medicinal products vs. botanicals: Clear borders are necessary

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The borders between herbal medicinal products (HMPs) and dietary supplements of herbal origin (botanicals) have recently been subject of political discussions within the EU. In this context the question arised, whether health claims based on traditional use should become possible for botanicals in the future. While herbal medicinal products have pharmacological effects and are used for therapy, relief, prevention or diagnosis of diseases, botanicals have a physiological effect and are primarily used for nutrition or health-related effects by healthy consumers. A transfer of the „traditional use“ principle from herbal medicinal products to dietary supplements, as currently under discussion, is not possible due to the fact that dietary supplements are not designated for the treatment of diseases. In addition, for herbal medicinal products there is typically a long, well documented tradition of their medicinal use for a well defined indication, as well as of their composition, posology and formulation. In contrast, for botanicals this information is usually lacking, as is the case also in other food items, which are often subject to frequent changes in manufacturing, composition, quality, and nutritional use. Therefore the GPT (Society for Phytotherapy, Germany) has submitted a statement to the German government reinforcing its position expressed previously and has argued against the introduction of "traditional" health claims for dietary supplements. The German federal government has followed this position when submitting its statement to the EU commission. Also in the future, the assessment of botanicals should be based on the question whether a relevant health-related effect has been proven by adequate scientific data and whether the dose resp. serving is equivalent to that consumed nutritionally. This is a necessary precondition for providing efficacious and safe products to patients and consumers in the future.

PN52 $GABA_A$ receptor binding assays of standardized *Leonurus cardiaca* and *L. japonicus* extracts as well as their isolated constituents

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A main traditional use of European *Leonurus cardiaca* L. and East Asian *Leonurus japonicus* Houtt. is the treatment of neurological disorders such as anxiety, depression, nervousness, and further as a sedative for insomnia. However, their mechanism of action is still under discussion. As anxiety and depressive disorders are increasingly being recognized as connected to dysfunctions of the GABA system, we investigated the *in vitro* effects of dry extracts of *L. cardiaca* and *L. japonicus* as well as five of their isolated constituents, the labdane-type diterpene isoleosibirin, the iridoid 7R-chloro-6-desoxy-harpagide, the phenylethanoid lavandulifolioside, and the N-containing compounds stachydrine and leonurine, on this type of neuronal receptor. Both extracts as well as the guanidino alkaloid leonurine inhibited the binding of [^{3}H]-SR95531 to $GABA_A$-receptors (GABA-site) in a concentration dependent manner. In contrast, binding to the benzodiazepine-site of the rat $GABA_A$ receptor had a 15 to 30 times lower binding affinity than to the GABA-site. In conclusion, the presented experiments provide strong hints that the neurological mechanism of action of *L. cardiaca* and *L. japonicus* is based on their interaction with the GABA-site of the $GABA_A$ receptor, while the benzodiazepine-site does most probably not contribute to this effect. In the case of *L. japonicus*, these effects can be at least partially explained by its leonurine constituent, whereas the active principle of *L. cardiaca*, which does not contain leonurine, is subject to further research as none of the other investigated isolated substances did display significant activity in the applied test system.

PN53 On the antispirochaetal activity of manoyloxides and carvacrol from the oleoresin labdanum of *Cistus creticus* L.

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Intrigued by testimonies of Saxon borreliosis self-help groups concerning considerable improvements of their symptoms by ingestion of *Cistus creticus* leaf preparations, we recently reported [1] on the growth inhibiting activity of extracts with different polarities and its volatile oil against *Borrelia burgdorferi s.s. in vitro*, determined by a bioassay guided procedure. Using GC-MS, the most active volatile oil was found to be dominated by manoyloxide type diterpenes and carvacrol. These are typical of the old pharmaceutical oleoresin labdanum [2], which is secreted from the leaf surface and traditionally harvested e.g. on Crete by brushing the shrubs. Consequently, we isolated and identified four manoyloxides, namely manoyloxide, epi-manoyloxide, 3-hydroxy-manoyloxide, 3-acetoxy-manoyloxide, from Cretan labdanum, which were tested for their antispirochaetal potential against *B. burgdorferi s.s. in vitro*, revealing epi-manoyloxide to exhibit by far the strongest individual effect, equal to the positive control amoxicilline. Furthermore, manoyloxide, the monoterpene carvacrol, and the diethyl ether soluble fraction of labdanum contribute to the antispirochaetal activity, while the other manoyloxides were less active. Isolated manoyloxides were further used as external standards for a GC-MS screening of labdanum samples from different origins, revealing exceptionally high contents of all analyzed manoyloxides in the samples of Cretan labdanum from *C. creticus*, while their contents in other East Mediterranean samples were several orders of magnitude lower. In Spanish labdanum samples from *Cistus ladanifer* (hot water extract!), mainly simple alkanes and only traces of the major antispirochaetal constituent epi-manoyloxide and of manoyloxide could be detected. **References:** [1] Hutschenreuther A, Birkemeyer C, Grötzinger K, Straubinger RK, Rauwald HW. Pharmazie. 2010 65:290 – 5. [2] Anastasaki T, Demetzos C, Perdetzoglou D, Gazouli M, Loukis A, Harvala C. Planta Med. 1999 65:735 – 9.

PN54 Screening of Kazakhstan plants useful in the treatment of diabetic foot syndrome

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One of the most serious complications of diabetes is the formation of diabetic foot syndrome. Neuropathy, vascular changes and infections are all contributors. Therefore, the aim of this study was to determine antimicrobial and antioxidant activities of crude extracts isolated from plants growing in Kazakhstan (*Vexibia alopecuroides* (L.) *Jakovl.; Rhodiola quadrifida* (Pall.) Fish. et Mey.; *Epilobium hirsutum* L.; *Rumex confertus* Wiild.), which can be used to create products for the treatment of diabetic foot syndrome. Antimicrobial activity was evaluated using NCCLS broth micro-dilution assays. 2,2-azinobis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt (ABTS) radical scavenging assay was used to determine the antioxidant activity. The results of the present investigation clearly indicate that the antibacterial and antifungal activities vary with the species of plant and only dichloromethane extracts produced favorable results in all assays. *Epilobium hirsutum* showed good activity against *Candida glabrata* (IC_{50} 39.7 µg/ml), *Rhodiola quadrifida* showed strong activity against *C. glabrata* (IC_{50} 2.9 µg/ml) and *Candida krusei* (IC_{50} 9.2 µg/ml), *Rumex confertus* showed good activity against *Staphylococcus aureus* (IC_{50} 10.8 µg/ml) and *Methicillin-resistant S. aureus* (IC_{50} 16.2 µg/ml) and *C. glabrata* (IC_{50} 2.9 µg/ml). Among the plant extracts, the highest antibacterial activity was seen from *Vexibia alopecuroides* against *S. aureus* (IC_{50} 3.05 µg/ml) and *Methicillin-resistant S. aureus* (IC_{50} 2.9 µg/ml), in spite of this fact *Vexibia alopecuroides* extract did not show any antioxidant activity. The other extracts showed a dose dependent ABTS scavenging activity. IC_{50} values were (6.6 µg/ml) *Epilobium hirsutum;* (4.5 µg/ml) *Rumex confertus;* (3.8 µg/ml) *Rhodiola quadrifida.* Thus, we have selected the most promising plant species for further investigation in the potential discovery of new natural bioactive compounds for treating diabetic foot syndrome.

PN55 *In vitro* absorption studies of saffron ingredients (*tr*-Crocin-1, *tr*-Crocetin) in the Caco-2 and blood-brain barrier model

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Saffron shows various effects on the central nervous system, e.g. improvement of long-term potentiation of learning and pronounced antidepressant properties, probably caused by NMDA-receptor activity and inhibition of glutamatergic synaptic transmission. Pharmacological effects have been investigated by *in vitro*, *ex vivo* and *in vivo* experiments, antidepressant properties were also confirmed by preliminary clinical trials. The pharmacological potential of this drug has been well studied but there is a lack of pharmacokinetic data. Since the main compounds of hydroethanolic saffron extracts are Crocins, glycosylated C-20 carotenoids, we decided to investigate the absorption properties by *in vitro* experiments with Crocin-1 and the aglycon Crocetin. Investigations were carried out on Caco-2 cell line, representing the intestinal barrier and on two other *in vitro* models representing the blood-brain barrier. The aim of the following study was to show intestinal permeation characteristics of Crocin-1 and Crocetin by Caco-2 cells and to demonstrate central nervous system availability of Crocetin by different blood-brain barrier models. The Caco-2 system was validated by quality assurance parameters as permeability of transport markers, transepithelial electrical resistance, and laser scanning microscopy. Permeation studies indicates that Crocin-1 was poorly absorbed (P_{app}= 2,33 *10^{-7} cm/s). In contrast high permeation rates were found for the aglycon Crocetin (P_{app}= 2,65 *10^{-5} cm/s). Availability of Crocetin in the central nervous system was deduced from findings of permeation through *in vitro* models of blood brain barrier (P_{app}= 1,48 *10^{-6} cm/s) and blood cerebrospinal barrier (P_{app}= 3,75 *10^{-6} cm/s). Based on these data it can be assumed that the glycosylated crocines are not bioavailable after oral administration, but after intestinal deglycosylation the aglycon Crocetin should be absorbed to the systemic compartment and should also permeate the blood-brain barrier.

PN56 The anti-inflammatory effects of a standardized extract of *Justicia pectoralis* (SEJP) on the antigen-elicited rat airway hyperresponsiveness involve changes in gene expression of canonical transient receptor proteins (TRPC)

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Justicia pectoralis Jacq. (Acanthaceae) has coumarin and umbelliferone as natural constituents. While the hydroalcoholic extract has relaxant properties in guinea-pig trachea, this plant is useful in folk medicine to treat respiratory diseases (*J Ethnopharmacol* 2000;70:151). Here, we evaluate the protective effects of SEJP against the development of the hyperresponsive phenotype of isolated Wistar rat trachea induced by antigenic challenge with ovalbumin (OVA). The standardized extract was prepared as described in *Phytother Res*. 2011;25:444. It was analyzed in HPLC and the content of coumarin and umbelliferone was 1.5 and 0.2 mg/ml extract, respectively. Antigen sensitization was achieved by the injection of OVA (10 mg/kg; i.p.) and antigen challenge occurred by inhalation of OVA (5 mg/ml) 14 days after the sensitization procedure. Tracheal rings of OVA-challenged group (OCG) were hyperresponsive showing significantly higher ($p < 0.05$) values of Emax to contractile stimuli due to acetylcholine (ACh; 10^{-8} to 10^{-3} M; Emax 1.0 ± 0.1 g) or KCl (10^{-3} to 10^{-1} M; Emax 0.9 ± 0.1 g) compared to their respective saline-challenged rats (SAL; 0.6 ± 0.1 g for ACh and 0.5 ± 0.1 g for KCl). OCG rats treated with SEJP (~ 150 mg coumarin/kg, p.o.) showed Emax values for ACh or KCl without significant difference compared to SAL ($p > 0.05$). SEJP treatment also reduced the hyperresponsiveness of thapsigargin-treated trachea submitted to extracellular Ca^{2+} restoration under Ca^{2+}-free medium (capacitative Ca^{2+} entry). SEJP also impaired ($p < 0.05$) the increased levels of TNFα and IL-1β in OCG. SEJP returned to basal levels ($p < 0.05$) the relative gene expression (by RT-PCR) of TRPC 1 and TRPC 5 in lung tissue, which were decreased and augmented, respectively, by the antigen challenge. Thus, SEJP has anti-inflammatory actions that prevent the development of tracheal hyperresponsiveness after antigen challenge in rats. Its effects appear to involve modulation in gene expression of TRPC proteins.

PN57 Bioprospecting diverse plant species for antiviral agents against upper respiratory tract infections

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Upper respiratory tract infections (URIs) are caused by various bacteria and viruses. In this work, we focused on the identification of natural constituents with antiviral activity on human rhinovirus (HRV2), a predominant cause of the common cold, influenza virus A and coxsackie virus (CVB3), both of them being responsible for more severe forms of URIs. Starting from a literature survey to explore knowledge from folk medicine, 66 plant species of interest were identified. The selection focuses on the flora of the Alpine region with Lamiaceae, Apiaceae and Asteraceae representing the most prominent families. Following the acquisition and collection of the selected plants, the obtained extracts were tested for their antiviral potential against the three viruses in cytopathic effect (CPE) inhibition assay. About 12% of the extracts had an IC_{50} lower than 50 µg/mL for influenza virus A/HK/68 in MDCK cells. A comparable ratio of active extracts was found for HRV2, and 8% of all extracts showed activity for CVB3, the latter two being tested in HeLa cells. Of all active extracts, 31% inhibited two and even 8% all three

distinct viruses. In addition to these experimental investigations, a structure-based computational approach was followed, aiming to support the interpretation of ethno-pharmacological data and guide isolation. As a preliminary result, five out of the 66 plant species were found to contain constituents that were predicted *in silico* to be active on influenza virus neuraminidase and nucleoprotein, as well as the HRV capsid protein 1. In the course of the experimental evaluation of these five extracts, all of them were confirmed to exhibit significant antiviral activity, affirming the potential of this bioprospecting approach in finding valuable starting points for target-based drug discovery. This work is supported by the Austrian Science Fund (FWF P24587 & P23051) and the European Social Fund (ESF and TMWAT Project 2011 FGR 0137).

PN58 Enhancing effect of *Thevetia peruviana* flower extract on trail induced apoptosis in human cervical cancer cells

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a potent stimulator of apoptosis. However, many cancer cells remain resistant to TRAIL. Currently, there is much interest in screening for natural products that can sensitize cancer cells to TRAIL-induced apoptosis for their use in combination with TRAIL. This study was conducted to investigate the effects of ethanolic extracts on TRAIL induced apoptosis in human cervical cancer (HeLa) cells by determinations of cell viability and apoptosis using WST-1 cell proliferating assay and immunoblot analysis, respectively. Pretreatment of HeLa cells with various concentrations (3 – 300 μg/mL) of flower ethanolic extracts of *T. peruviana* enhanced TRAIL induced antiproliferation in HeLa cells. Extracts at concentration of 10, 30, 100 and 300 μg/mL together with TRAIL significantly decreased viability of HeLa cells compared to that treated only with TRAIL. Compared to HeLa cells treated with *T. peruviana* extract alone, only extract at concentration of 10 μg/mL together with TRAIL strongly decreased cancer cell viability. The extract at concentration of 10 μg/mL strongly promoted TRAIL induced cleavage of caspase-3 and PARP-1. The results suggested that ethanolic extracts from flowers of *Thevetia peruviana* show an enhancing effect on TRAIL induced apoptosis in HeLa cells by activation of the caspase cascade.

PN59 Free radical scavenging activity of extracts from *Cajanus cajan* (L.) Millsp.

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The antioxidant activity of Pigeon pea *(Cajanus cajan*) has been investigated mostly in leaf and pod extracts. The research was to investigate the potency of various plant parts including root, stem, leaf and seed from pigeon pea collected from Khonkaen Province, Thailand. Different solvents, including ethanol, dichloromethane and water, have been used to prepare plant extracts. The extracts were analyzed for total phenolic content and antioxidant capacities. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, 2,2'-azinobiz(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and Ferric reducing antioxidant power assay (FRAP assay) were used to analyze the antioxidant capacity. The total phenolic contents in ethanol, dichloromethane and water extracts were 0.870 – 4.408, 0.222 – 2.833 and 0.778 – 3.972 mg GAE/1 mg extract, respectively. Ethanol extract from stems showed the highest total phenolic content (4.408 mg GAE/1 mg extract) and also exhibited high antioxidant capacity by ABTS assay (IC_{50}= 1.119 mg/mL). The highest antioxidant capacity (IC_{50}= 0.141 mg/mL) examined by DPPH assay was observed in the seed ethanol extracts. However, the highest antioxidant capacity by FRAP assay (IC_{50}= 26.653 μg vitamin/1 mg extracts) was found in water leaf extracts. The results indicated that the ethanol extracts from *Cajanus cajan* showed the highest antioxidant capacity in comparison to those from other solvents, suggesting their potency for nutraceutical and therapeutic applications.

PN60 Validation of plant materials used by resource-limited livestock farmers for ethno-veterinary medicine in the Eastern Cape province, South Africa

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Traditional medicine has a significant role in the animal health care system in Africa and resource limited rural communities. There has been much documentation of the plant materials used in ethno veterinary medicine world-wide, but limited validation to determine the effectiveness and potential toxicity of the materials used. Over the years, our team has focused on documenting, assessing effectiveness and potential toxicity of plant materials used by farmers in treatment and control of external and internal parasites of livestock. Their antimicrobial, antioxidant, analgesic and anti-inflammatory properties were also assessed. This was achieved using qualitative data collection. Validation of the plant materials was done using in vitro assays (tick, flea and egg hatch for helminthes) and for in vivo assessment goats, cattle and the rat model were used.

sPlants screened	Example of medicinal properties	Dose range
Aloe ferox Mill	Antioxidant Antibacterial	0.2 mg/ml 0.1 – 5.0 mg/ml
Elephantorrhiza elephantina (Burch.) Skeels	Antihelminthic	0.625 – 25 mg/ml
Leonotis leonurus (L) R.Br.	Anti-inflammatory *and* Analgesic	25 – 75 mg/ml
Ptaeroxylon obliquum, (Thunb.) Radlk *Lantana, camara* L *Tagetes minuta* L	Acaricidal	10 – 30% (w/v)
Agave sisalana Perrine	Anti-inflammatory and Analgesic	50 – 400 mg/kg BWt
Ptaeroxylon obliquum (Thunb.) Radlk *Calpurnia aurea* (Aiton) Benth	Insecticidal	10 – 100% (w/v)

Plants evaluated for toxicity were found to be safe at levels of 100 mg/ml and below. The results showed some of the materials to be as effective as the conventional remedies, whereas others were not. These finds will contribute to the body of knowledge to be used in the production of an organic animal. **Reference:** [1] Maphosa V Masika PJ 2012. In vivo validation of Aloe ferox (Mill). Elephantorrhiza elephantina Bruch. Skeels. and Leonotis leonurus (L) R. BR as potential anthelminthics and anti-protozoals against mixed infections of gastrointestinal nematodes in goats. Parasitol Res 110:103 – 108

PN61 Development and characterization of microparticles from *Paullinia cupana* var. *sorbilis* (Guaraná) semipurified extract produced by spray drying

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Introduction: Guaraná is well-known for its dietary and pharmaceutical potential and the semipurified extract of Guaraná (EPA) show antidepressant and panicolytic effects [1 – 2]. However, their low solubility, bioavailability and stability limit their use as components for pharmaceutical agents. For drug delivery, EPA in a micro/nanoparticle form is desirable for their optimized stability. **Methods:** In this study, EPA microparticles produced by spray drying process using a combination of maltodextrin (MD) and arabic gum (AG) as coating polymer. The drug content by high performance liquid chromatography (HPLC) according to catechin and epicatechin content and antioxidant activity (DPPH test) were evaluated. *In vitro* dissolution tests, using flow cell dissolution equipment, were carried out to investigate the influence of formulative parameters on EPA release from the microparticles. **Results:** The spray drying technique and the process conditions selected have given satisfying encapsulation efficiency (80 – 100%) and product yield (55 – 60%). The microencapsulation has improved the technological characteristics of the powders and has preserved the antioxidant properties of EPA. The scanning electron microscopy (SEM) micrographs revealed that EPA quantity had a significant influence on the microparticles (Figure 1).

Conclusion: This spray drying technique can be used as an efficient and economic approach to produce EPA microparticles.

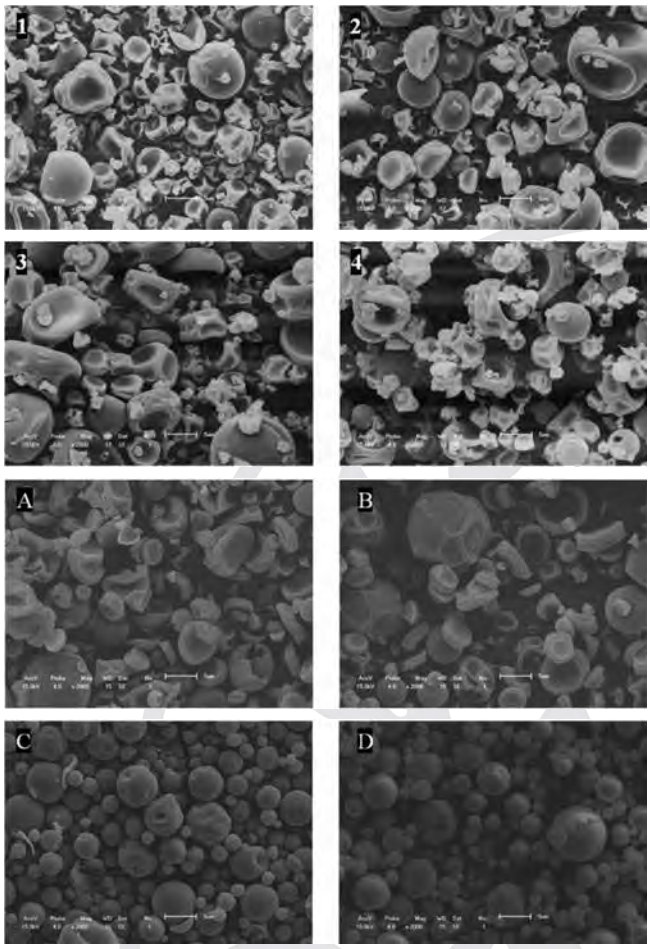

Fig. 1: SEM photomicrographs of the microparticles (2000X). 1, 2, 3 and 4: formulations of samples 1, 3, 12 and 13 respectively without EPA; A, B, C and D: samples 1, 3, 12 and 13 respectively.

Acknowledgements: Fundação Araucária, CNPq, CAPES **References:** [1] Otobone, F.J., Sanches, A.C.C, Nagae, R.L., Martins, J.V.C., Sela, V.R., Mello, J.C.P., Audi, E.A. 2007. Phytotherapy Research 21, 531 – 535. [2] Roncon, C.M., de Almeida, C.B., Klein, T., Mello, J.C.P., Audi, E.A. 2011. Planta Medica 77, 236 – 241.

PN62 Mutagenicity and antioxidant activity of *Croton antisyphiliticus* Mart

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Croton antisyphiliticus is used in folk medicine of Brazil as antisyphilitic, diuretic and anti-inflammatory. Toxicological and pharmacological studies are scarce, so this study aimed to evaluate mutagenic and antioxidant activity of methanolic extract and fractions of vegetative parts. To evaluate its genotoxicity it was determined the frequency of micronucleus in bone marrow of rats. The antioxidant activity was determined by DPPH test, and iron chelator. Additionally we determined the levels of total phenols and flavonoids. All fractions obtained showed no mutagenic activity and showed significantly lower micronucleus frequency compared to the negative control (water = 0.39%). The antioxidant activity of the methanolic extract was evaluated in 81% in DPPH test and 77.8% for the test of iron ion chelator. The methanolic extract and its fractions showed the presence of phenols and flavonoids. It was possible to conclude that the methanolic extract and its fractions show no mutagenic activity, and the high antioxidant activity may be related to different phenolics compounds, especially the presence of flavonoids.

PN63 Mucin-polysaccharides interactions: In search of a nanobioplatform for Helicobacter pylori therapy

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Mucoadhesion is defined as the ability of a material to adhere to the mucosal surface of tissues for an extended period of time [1]. A mucoadhesive drug delivery system can improve the controlled delivery and optimize the bioavailability of drugs for a prolonged period of time. Polysaccharides are among the most versatile families of macromolecules and their potential use in transmucosal drug delivery application is fully recognized [2,3]. The mucoadhesion of various polysaccharide families of varying structural characteristics and natural sources was explored by a sensitive micorviscosimetric method in order to contribute to a more rational design of mucoadhesive polysaccharide-based nanoparticles as potential carriers of compounds that interfere either with the adhesion or with the avability of essential nutrients of the *H. pylori* in the stomach mucosa. Here we show how deviation in viscosity of mixed solutions of polysaccharides and soluble fraction of mucin with respect to the viscosity of the original stock solutions can be considered as diagnostic of molecular associative interactions [4]. In the light of the results obtained we could observe a strong correlation between interaction and the ability of polysaccharide coils to contract in the presence of salt (i.e. chain flexibility). Moreover, the "more neutral" polysaccharides such as dextran, mesquite gum and a bacterial exopolysaccharide from *Streptococcus thermophilus,* showed no interaction with mucin. The adopted approach is perhaps an oversimplification of the mucoadhesion behavior *in vivo* during the interaction of polysaccharides with the mucous epithelia. However, it has allowed gaining further insight into the possible mechanisms underlying the mucoadhesion phenomena. **References:** [1] Liu, Z.H., et al. *Advanced Drug Delivery Reviews*, 2008. **60**(15) [2] Smart, J.D. *Advanced Drug Delivery Reviews*, 2005. **57**(11) [3] Sandra, K. *American Chemical Society*, 2009 [4] Hassan, E.E. and J.M. Gallo. *Pharmaceutical Research*, 1990. **7**(5)

PN64 Formulation of herbal drugs

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Among the newly studied medicinal plants is *Zizyphus spina-christi* L. which was investigated for its antidiabetic activity against type II diabetes. Formulation of different leaf extracts and standardization of the chosen extract using its major identified saponin as active marker was performed using HPLC. Evaluation of anti-diabetic activity of extract (200 mg/kg b.w.), plain and formulated in STZ-diabetic rats was performed. Percentage yield of extracts, marker yield and antihyperglycemic potencies, depending on seasonal variation were investigated as well as chemical stability, study of storage conditions, shelf life T90 prediction by accelerated studies. The marked increased use of complementary and alternative medicine has augmented the interest in Phytopharmaceuticals. Researches on medicinal plants were performed to introduce new medicinal plants formulation into the market. A second idea was applied using *Nigella sativa* L. through a bioactivity guided fractionation of different seed waste extracts to evaluate their hepatoprotective activities by both biochemical and histopathological investigations. The aqueous extract attenuated the CCl_4-induced liver damage. Fractionated of this extract into its component, protein, saponin and polyphenol fractions were evaluated by analytical procedures. It is concluded that the protein fraction exhibited promising hepatoprotective effect in the management of different liver disorders. This aqueous ex-

tract was formulated into soft uncoated gelatin capsules (Su1-Su6) in a dose of 200 mg/kg b. w. and the formulations were evaluated for their immunostimmulant and hepatoprotective activities. Formula Su3 was chosen being of highest activity and optimum release to be coated with two film-coating solutions (Sc3) for site-specific delivery and sustaining the release. Quality control of formulated extract (Sc3: Eu.S-100) was performed using protein as active marker.

PN65 Sequencing of alliinase gene from *Allium stipitatum* REGEL subgenus *Melanocrommyum*

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The genus *Allium* contains about 800 species divided into different subgenera [1]. All species have an enzyme called alliinase, which catalyzes the cleavage of cysteine sulfoxides forming bioactive aroma compounds [2]. Common species like *Allium sativum* or *Allium cepa* are well characterized by secondary plant compounds as well as their alliinases [3,4]. *Allium stipitatum* is well established as a condiment in Central Asian cuisine. However no research has been done on its alliinase yet. Besides, *A. stipitatum* contains a unique pyridinyl cysteine N-oxide, which is subjected to alliinase [5]. *A. stipitatum* and *A. sativum* display a different protein pattern on SDS-gels (Fig.). A protein extract of *A. sativum* has a high amount of alliinase (monomers at 50 kDa), whereas *A. stipitatum*'s protein extracts only show a faint protein spot up there. Highly conserved sequences of alliinase genes were used to design primers for amplification of the corresponding alliinase gene in *A. stipitatum* via PCR. Thus it was possible to obtain a part of an alliinase gene. Using the method of restriction enzyme site-directed amplification [6], the gene was extended to the flanking regions leading to the whole alliinase gene of *A. stipitatum*, which will be the first complete gene of subgenus *Melanocrommyum* in data bases. Investigation of mRNA proved the gene expression in *A. stipitatum* bulbs. The gene consists of five exons and four introns. Despite the wide differences in protein expression scheme of *A. stipitatum* and *A. sativum*, their alliinases amino acid sequences are 83% alike and only differ four amino acids in length.

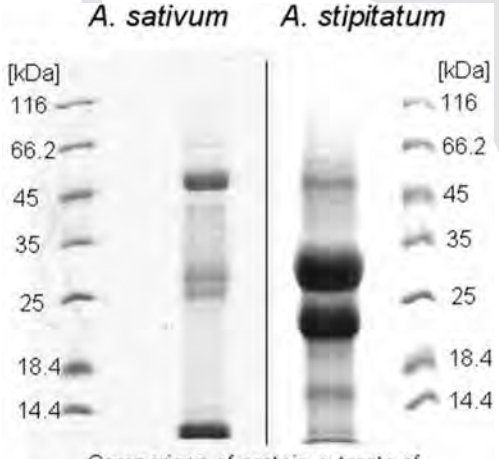

Fig. 1 Comparison of protein extracts of *Allium sativum* and *Allium stipitatum*

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PN66 The anti-cancer effect of the pentane fraction of *Daucus carota* oil extract is mediated through cell cycle arrest and an increase in apoptosis

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Daucus carota L. ssp. *carota* (Apiacea) is used in traditional medicine in Lebanon and in different regions throughout the world. Previous studies from our laboratory demonstrated the *in vitro* anticancer activities of *Daucus carota* oil extract (DCOE) on colon and breast human cancer cell lines. In the current study, we aimed at fractionating the *Daucus carota* oil extract (DCOE) and analyzing the *in vitro* anticancer activities of the obtained fractions. The pentane fraction (PF) proved to have a highly cytotoxic effect on cancer cells. Cell cycle analysis showed that the cells treated with 25 mg/ml of the pentane fraction completely stopped cycling and had a substantial decrease in the number of cells in G2. PI/Annexin staining also showed an increase in apoptosis in cells treated with PF. Western blot analysis of several apoptotic proteins also proved a direct activation of PF of the apoptotic pathway in cancer cells. The fact that the drug affected both apoptosis and the cell cycle suggested that it is exerting its effect on an effective pathway such as the PI3K pathway or the MAPK pathway. We treated the cells with PF and performed western blots looking at p-Akt and p-ERK. We indeed determined that the PF treatment led to a decrease in p-ERK as compared to total ERK levels, whereas the level of phosphorylated Akt was not affected. This showed that the anti-cancer effect of the PF fraction of wild carrot is due to its inhibition of the ERK pathway which leads to cell cycle arrest and an increase in apoptosis in the tested cancer cells.

PN67 Anti-inflammatory effect of the methanol, ethyl acetate and chloroform extracts of *Tragopogon porrifolius* aerial parts

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Tragopogon porrifolius L. (Asteraceae) is an edible plant widely used in the Lebanese folk medicine. The present study investigates the acute anti-inflammatory effect of the methanolic, ethyl acetate and chloroform extracts of the aerial parts of T. porrifolius and the chemical analysis of the extracts using GC-MS technique. Acute anti-inflammatory activity was tested using the carrageenan-induced paw edema model in mice. Doses of 250 mg/kg, 100 mg/kg, and 50 mg/kg body weight of each extract were administered intraperitoneally prior to carrageenan treatment and measurements of paw thickness were taken at 0 and 4 hours. The methanolic extract exhibited maximum anti-inflammatory activity (60%; 250 mg/kg), followed by ethyl acetate (36%; 250 mg/kg), and chloroform (29%; 100 mg/kg) extract, when compared with the untreated control group. The inhibition of paw edema by the methanolic extract was comparable in effect to that of diclofenac (10 mg/kg) treatment (69%). GC-MS analysis revealed the presence of β-amyrin acetate in the three extracts: 20.9% in the methanolic (major), 9.52% in the ethyl acetate, and 7.42% in the chloroform extracts. β-amyrin acetate has been reported in the literature to possess anti-inflammatory and nociceptive activities and might be partly responsible for anti-inflammatory effects of the three *Tragopogon porrifolius* extracts. *Acknowledgments:* Mr. Jean Karam.

PN68 Antimicrobial and cytotoxic properties of semisynthetic betulin derivatives

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Betulin (lup-20(29)-ene-3β, 28-diol) is an abundant naturally occurring triterpene, which is the principal extractive of outer birch bark. A collection of 65 structurally diverse semisynthetic betulin derivatives was screened against six different microbes, *Enterobacter aerogenes*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa, Staphylococcus aureus* and a fungal strain *Candida albicans*, using microdilution assays based on CLSI and EUCAST guidelines. Primary antimicrobial screening led to the identification of six compounds showing promising antimicrobial properties (inhibition > 70% against one or more microbial strains). According to the dose-response results, the betulin derivative SC047 was the most active, showing MIC of 6.25 μM against two Gram-positive bacteria, *E. faecalis* and *S. aureus*. However, the activity of this compound was affected by albumin binding, which was demonstrated by the loss of activity in the host-pathogen co-culture assay as well as in the antibacterial assay in the presence of increased concentration of albumin. Furthermore, cytotoxicity assessment based on ATP measurement of human hepatocyte cell culture after 24 h exposure to the compounds, showed that compounds SC013 and SC062 displayed cytotoxicity towards hepatocytes, showing IC_{50} of 25.0 μM, and 15.8 μM, respectively. In addition, the IC_{50} value of 55.9 μM for compound SC047 was determined. The current study presents an insight into using betulin scaffold for developing derivatives with antibacterial potential, and furthermore the necessity of in-depth analysis of found actives through selectivity profiling and functional characterization. Moreover, the results demonstrate the importance of taking a multidimensional approach when studying biological activities of natural compounds and their derivatives.

PN69 Reporting of adverse reactions from plant-based products in Norway from 2003 to 2012

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Purpose Since 2003 the Regional Medicines Information and Pharmacovigilance centre (RELIS) has handled reports on adverse reactions from medicines and dietary supplements from health care professionals. This is the first review of the data concerning the plant-based products, and highlights the trends related to the reporting frequency, categories of health care professionals that are reporting, the side effects most frequently reported, the severity of the reactions and patient characteristics. **Materials and methods** The data includes all reports of plant-based products in the Norwegian safety database from 2003 to 2012.

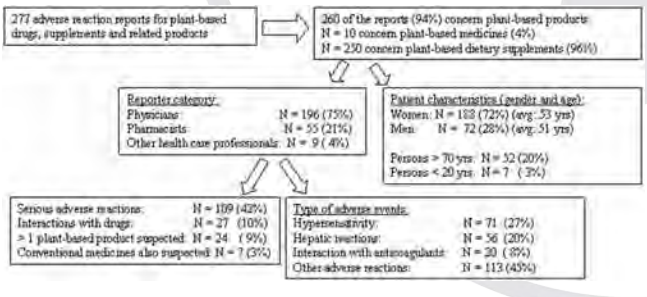

Fig. 1: Characteristics of adverse event reports for plant-based products for the period 2003 – 2012 in the Norwegian database for adverse drug reactions.

Results and conclusion Since 2003 RELIS has received 260 reports on adverse reactions associated with plant-based products. Of these, 96% concern plant-based dietary supplements. Of the 10 reports on plant-based medicines, all were reported by pharmacists, and none were classified as severe. In the last three years, the reporting rate has decreased. In 2012, pharmacists did not report any adverse reactions from plant-based products. Hypersensitivity reactions, hepatic events and interactions with anticoagulants are the most commonly reported adverse effects for plant-based products (Fig. 1). Increased focus on adverse reactions reporting can generate important knowledge about such products.

PN70 Glucose lowering effect *in vitro* of *Astraeus odoratus* extracts

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Astraeus odoratus C. Phosri, R. Watling, M.P. Martin & A.J.S. Whalley (Diplocystaceae) is a mushroom distributed in the Northeastern part of Thailand and has been widely used as food. Here we investigate the activity *in vitro* which is related to blood glucose lowering. The *A. odoratus* aqueous extract (yield 2.1% dry weight), and 95% EtOH extract (yield 2.4% dry weight) and its fractions, were assayed for alpha glucosidase inhibition in 96-well plates; the glucose uptake stimulation was determined by using 2-deoxy glucose assay in L6 myotube cells. Fractionation of the 95% EtOH extract was done by a Diaion HP20 column to obtain 5 fractions (H_2O, 25% MeOH, 50% MeOH, 75% MeOH, and 100% MeOH respectively). The results indicated that *A. odoratus* extracts possessed alpha glucosidase inhibition with IC_{50} of 98.9 μg/mL for the aqueous extract and IC_{50} of 63.3 μg/mL for the 95% EtOH extract. The glucose uptake stimulation into L6 myotubes of the aqueous extract was found to be 22% at the concentration of 400 μg/mLwhile the 95% EtOH extract was inactive. Fractionation of the 95% EtOH extract led to increasing alpha glucosidase inhibition potency by the 75% MeOH fraction with IC_{50} 23.4 μg/mL while the IC_{50} of acarbose was 3.6 mg/mL. For the glucose uptake stimulation, the 100% MeOH fraction (400 μg/mL) was the active fraction with increasing potency to 42% stimulation while the positive control 500 nM insulin provided 100% stimulation. It can be concluded that *A. odoratus* extracts mainly affected the postprandial blood glucose reduction rather than the glucose uptake stimulation into the muscle cells. In addition, the fractionation of 95%EtOH extract led to the increasing of glucose lowering effect.

PN71 Antidiabetic and biochemical effect of the different fractions of methanol extract of *Detarium microcarpum* (Fabaceae) Guill and Perr stem bark on normal and alloxan induced diabetic rats

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In this study, anti-diabetic activity of n-hexane fraction (HF) chloroform fraction (CF), diethyl ether fraction (DEF), ethyl acetate fraction (EAF) and methanol fraction (MF) of the methanol extract of *Detarium microcarpum* stem bark was investigated in normal and alloxan-induced diabetic rats. The fractions were screened for phytochemicals using standard methods. The acute toxicity (LD_{50}) of the fractions was determined in mice. The fractions were subjected to anti-diabetic study in alloxan-induced diabetic rats at two dose levels, 200 and 400 mg/kg. Diabetes was induced by a single ip injection of 120 mg/kg of alloxan monohydrate and glucose level was analyzed as indices of diabetes. Biochemical parameters, including glucose, urea, creatinine, serum cholesterol, serum triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) haemoglobin and glycosylated haemoglobin were also assessed.The acute toxicity test showed that the fractions were safe at doses of up to 5 g/kg. The results indicated that intraperitoneal injection of CF, DEF, EAF and MF reversed the effect of alloxan in rats by different degrees. The anti-diabetic potency of fractions was in the order MF > CF >EAF > DEF and the activity of MF was comparable to that of the standard, glibenclamide (5 mg/kg). Treatment of diabetic rats with fractions of the methanol extract restored the elevated biochemical parameters significantly ($p < 0.05$) to the normal level. Histological studies showed a degenerative effect on the pancreatic islet cells of diabetic rats. The results suggested restorative (protective) effect of the MF on the pancreatic islet cells. The results of this study justify the use of this plant stem bark as traditional treatment for diabetes mellitus.

PN72 Pomegranate suppresses PGE_2 production and COX-2 expression in IL-1β-stimulated SK-N-SH neuronal cells: implications for Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia in the elderly and several reports have shown that neuroinflammatory processes contribute to the pathogenesis and progression of AD. Although a number of drugs have been developed for use, they have been shown to produce many side effects with limited therapeutic benefits. Recently, naturally occurring dietary substances have received considerable attention as alternative for AD therapy. Pomegranate (*Punica granatum*) is consumed globally as a fruit and has been shown to possess powerful antioxidant, anti-carcinogenic and anti-inflammatory properties. In the present study, we have investigated the effects of a lyophilised extract of pomegranate juice (PWE) in IL-1β-stimulated SK-N-SH neuronal cells. Cultured SK-N-SH cells were stimulated with IL-1β in the presence or absence of PWE (25 – 200 μg/ml) for 24 h. Results show that PWE produced a dose-dependent suppression of PGE_2 production and COX-2 protein expression when compared with IL-1β control. Furthermore, PWE (25 – 200 μg/ml) dose-dependently inhibited IL-1β-induced nuclear translocation of the NF-κBp65 subunit and IκB phosphorylation in SK-N-SH cells. Consistent with its anti-neuroinflammatory actions, PWE inhibited IL-1β-induced Aβ1 – 42 generation and expression of β-secretase (BACE-1). Taken together, it is suggested that pomegranate fruit may be useful in preventing the development and progression of AD. **Acknowledgement:** This study was funded in part by the Alexander von Humboldt Foundation.

PN73 Effects of sesquiterpene lactones on lipoxygenase activity

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Sesquiterpene lactones (STL) are natural compounds with a wide structural variety and a broad array of biological activities. Some of these activities are related to the capacity of STL to modulate different enzymes by inhibition or induction of their activities[1]. Lipoxygenase (LOX) is a ubiquitous enzyme that occurs in microorganisms, animals and plants. In these living organisms, LOX may display different functions depending on the products formed as a result of its reaction with fatty acids. In plants, for example, LOX acts on growth, development, senescence and defense by conversion of linoleic acid to jasmonic acid derivatives[2]. In animals, LOX is mainly related to the inflammatory process by conversion of the arachidonic acid into eicosanoids. Thus, the aim of this work was to evaluate the potential of STL in the modulation of the LOX activities. So far, about 30 STL were isolated from different species of the family Asteraceae and 26 of them were tested in an *in vitro* assay for the inhibition of the 5-LOX isoform. The results show that 10 STL were active by inhibiting the enzyme at relatively low concentrations. The most effectives of them were chamisellin (IC_{50}= 0.18 μg/mL), polimatin B (IC_{50}= 1.91 μg/mL) and fluctuanin (IC_{50}= 2.02 μg/mL). These results suggest the STL have a potential use in the modulation of the LOX activity. Further studies will be carried out aiming to analyze structure-activity relationships.

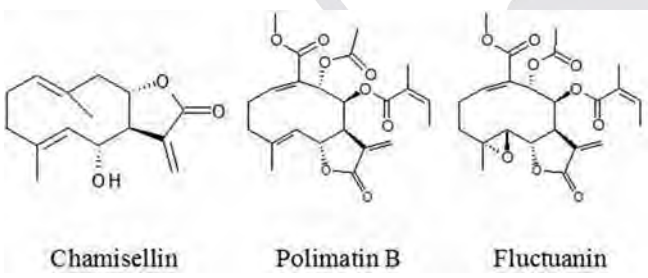

Fig. 1: The most effective STL in the 5-LOX inhibition assay

Acknowledgements: FAPESP. **References:** [1] Tornhamre S. et al. 2001. Biochem. Pharmacol. 62: 903. [2] Feussner I. et al. 1995. Proc. Natl. Acad. Sci. 92: 11849.

PN74 Spray dried *Petiveria alliacea* extracts: standardization and evaluation of the antimicrobial activity

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Petiveria alliacea L. (Phytolaccacea) is an herbaceous plant of great importance in folk medicine. Methabolites isolated and reported for *P. alliacea* include flavonoids, triterpenes, steroids, coumarin, and several sulfur-containing amino acids in the roots; as well as S-benzylcysteine sulfoxides, and S-(2-hydroxyethyl) cysteine sulfoxides. This study aimed to evaluate the in vitro antifungal activity of spray dried extracts of *P. alliacea* (leaves). Spray dried extracts were produced from hydro-ethanolic extract 70% (v/v) obtained by dynamic maceration of leaves. Maltodextrin and colloidal silicon dioxide at proportion of 5 and 2.5% respectively were added to the concentrated extract prior to drying (spray dryer model SD-05 LabPlant), at temperatures from 87 to 154 °C and extract feed rate of 6 g/min. The dried extracts were monitored for their antifungal activity by microdilution broth (M27-A2, CLSI) using standard ATCC strains: *C. parapsilosis* (2209), *C. albicans* (90028), *C. kefyr* (6258) and by concentration of total polysulphides expressed as dibenzyl trisulphide (DTS) using a UV-vis method. The antifungal activity was determined by the minimum fungicidal concentration (MFC) using fluconazole (0.125 – 16 μg/ml) as reference control. The hydro-ethanolic extract presented a MFC of 246 μg/ml for *C. albicans*, *C. parapsilosis* and *C. kefyr*. The dried extracts obtained at temperatures of 87 and 154 °C exhibited antifungal activity at concentration of 143 μg/ml for all strains tested, while the extract obtained at 120 °C presented MFC between 142 – 427 μg/mL. The concentrated extract showed a value of 11.7% g/g DTS while the dried extracts (temperatures 87 – 154 °C) showed an average value of 11.5% g/g. The results showed that the feed composition and drying conditions did not affect the DTS content. The dried product showed antifungal activity compared with the original concentrated extract, which presented MFC ranging from 82 μg/mL to 246 μg/mL, depending on the strains tested.

PN75 In vitro antioxidant potential of spray-dried *Psidium guajava* L. leaves extracts

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Psidium guajava (Myrtaceae), popularly known as guava, is widely used in traditional medicine due to its anti-inflammatory, antimicrobial and antioxidant properties. The aim of this research was to study Psidium guajava extract spray-drying in order to obtain powders with good antioxidant properties and stability attributes. The experiments were carried out in a SD 05 LabPlant spray dryer using maltodextrin, Arabic gum, colloidal silicon dioxide and β-cyclodextrin at concentration of 80% relative to solids content. The ABTS, DPPH, FRAP and ORAC assays were used for estimating antioxidant activity (AA) from guava extracts spray dried preparations (SDP). The chemical markers were evaluated through determination of the total phenolic (TP) and flavonoid (TF) content. Averaged AA [μM Trolox equivalent (TE)/g] were 3,296, and 1,913 as determined by the ABTS and ORAC respectively. The IC50 of the SDP ranged from 7.96 to 8.11 μg/mL using the DPPH· method. The FRAP values ranged from 4,210 to 4,540. Pearson's correlation coefficients were calculated in order to determine the association between antioxidant activity assays, total phenolics and flavonoids content. AA determined by DPPH assay had strong negative correlation with TF (r = -0.976), TP (r = -0.980). The FRAP assay showed a strong positive relationship comparing to ORAC (r = 0.965). FT was strongly positive correlated with PT (r = 0.947). In conclusion, the combination of methods was useful to confirm the antioxidant potential of guava SDP which displayed significant antioxidant activities.

PN77 Inhibition of NO-production by *Lonicera* extracts

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The dried and powdered leaves from *Lonicera* species (*L. dasystyla, L. hypoglauca, L. confusa, L. macranthoides, L. fulvotomentosa, L. bournei, L. ligustrina, L. similis, L. macrantha, L. rytidophylla, L. japonica, L. pampaninii, L. acuminata*) were extracted with ethanol using Accelerated Solvent Extraction (ASE). The extracts were tested for inhibitory effects on NO-production in LPS/IFN-γ stimulated RAW 264.7 mouse macrophages. Quantification of produced NO was carried out using the Griess-staining method[1]. Two of the samples showed a very good inhibitory effect on NO production with inhibition values of 87,79% (± 9,50) respectively 85,76% (± 6,67) whereas the positive control (L-NMMA) showed an inhibition value of 51,76% (± 6,39). LC-MS analyses have been performed with the two active extracts and it was shown that they have very similar LC-MS profiles, but distinct from the others. Identification of the active compounds is in progress. **References:** [1] Konkimalla V.B. et al. (2008) Nitric Oxide 19, 184 – 191. **Acknowledgements:** We gratefully acknowledge the funding provided by the Austrian Science Fund (FWF) for project S 10705-B13 within the NFN Drugs from Nature Targeting Inflammation

PN78 Determination of melatonin content in traditional Thai herbal remedies used as sleeping aids

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Melatonin content was screened in seven herbs used as sleeping aids in Thai traditional medicine. These herbs are *Piper nigrum* L, *Sesbania glandiflora* (L.) Desv., *Sesbania sesban*, *Senna tora* (L.) Roxb, *Moringa oleifera, Momordica charantia* L. and *Baccaurea ramiflora* Lour. Dried herbs were extracted by sonication in methanol for 6 hours at room temperature, then melatonin was purified by C 18 solid phase extraction. Melatonin was quantified by RP-C 18 HPLC with fluorescent detection. The highest melatonin content was from *Piper nigrum* extract (1092.7 ng/g of dry sample weight). Melatonin contents in extracts of B. ramiflora, S. glandiflora, M. charantia, S. tora and S. sesban were 43.2, 26.3, 21.4, 10.5 and 8.7 ng/g of dry sample weight, respectively. Melatonin peak was absent in the extract of *M. oleifera*. Melatonin identification was confirmed by ELISA. **References:** [1] Ansari M, Rafiee K, Yasa N, Vardasbi S, Naimi SM, Nowrouzi A: Measurement of melatonin in alcoholic and hot water extracts of Tanacetum parthenium, Tripleurospermum disciforme and Viola odorata. *Daru* 2010, **18:**173 – 178. [2] Sangkasat A JJ, Johns NP, Priprem A: Validation of Method for Determination Of Melatonin In Human Plasma By HPLC-Fluorescence Detector. *Laos Journal of Science* 2011, **2:**53 – 57

PN79 Immunomodulatory activity of *Dioscorea membranacea* Pierre and its compound

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Roots of *Dioscorea membranacea* Pierre (DM) was used as an ingredient of Thai traditional medicine for treatment cancer and AIDs patients [1]. The aim of this study was to investigate the effect of DM extracts, and its compound on natural killer (NK) cells activity and lymphocyte proliferation activity. Immunomodulatory activity was investigated by using peripheral blood mononuclear cells (PBMCs) from healthy donors. NK cell activity was performed by chromium release assay which used PBMCs as effector cells. The target cells were used as K562 cells which were labeled with chromium. Lymphocyte proliferation were determined by ^{3}H-thymidine uptake [2]. The degree of activation was expressed as stimulation index. The results showed that the ethanolic extract of DM significantly activated NK cells activity at concentration of 10, 100 ng/ml and 1 μg/ml as 1.36, 1.41 and 1.44 times by comparison with control. The water extract significantly activated NK cells activity at concentration of 10 ng/ml- 100 μg/ml as 1.16 – 1.52 times. The activated lymphocyte proliferation at concentration of 1 ng/ml-100 μg/ml as 1.24 – 1.8 times and significant different when compared with control. For its isolated compound, dioscorealide B [3], significantly decreased NK cells activity and lymphocyte proliferation. By conclusion, both extracts ofDM activated NK cells activity and lymphocyte proliferation but its compound, dioscorealide B decreased NK cells activity and lymphocyte proliferation. Our results support using DM extracts in Thai traditional medicine for increasing immune function in cancer and AIDs patients. **References:** [1] Itharat, A. et al.,. 1998. Wisdom of Southern Thai traditional doctors. Research report of Prince of Songkla University. Songkhla: Prince of Songkla University.126. [2] Sriwanthana, B. and Chavalittumrong, P. 2001. Journal of Ethnopharmacology. 76, 125 – 129. [3] Itharat, A. et al., 2003. Org Lett. 5, 2879 – 2882.

PN80 Flavonoid accumulation in *Hypericum androsaemum* cell cultures: involvement of cAMP/PKA signaling

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Hypericum androsaemum L. is a perennial herb growing wild in damp or shady areas throughout Eurasia. Leaf infusions have been used traditionally for its diuretic, cholagogue, and hepatoprotective properties [1]. Recent research has also shown that plant water extracts exhibit antitumor [2], antioxidant and anti-acetylcholinesterase activities [3]. Cell cultures established from hypocotyl-derived callus of *H. androsaemum* were reported [4] to accumulate small amounts of flavonoids, with maximum levels being reached on day 14. In a later study [5], it was found that treatment of 11-day-old cultures for 72 h with 15 mM $CaCl_2$ or 5 μM calcium ionophore A23187 induced a significant increase in the accumulation of flavonoids and in the activity of phenylalanine ammonia-lyase (PAL), a key regulatory enzyme of phenylpropanoid metabolism. Given that Ca^{2+} and cAMP act in a concerted fashion to regulate a variety of cellular processes, similar experiments were performed using either 100 μM dibutyryl-cAMP, a membrane-permeable cAMP analog, or 100 μM IBMX, a cNMP phosphodiesterase inhibitor. Treatments with these cAMP-modulating agents also caused a marked increase in both PAL activity and flavonoid content of cells recorded on day 14. In addition, pretreatment of cultures with 50 μM Rp-cAMPS, a specific inhibitor of cAMP-dependent protein kinase A (PKA), completely abolished the Ca^{2+} induced rise in flavonoid contents and concomitantly reduced the enhancement of PAL activity by about 60%. Collectively, these data suggest that cAMP/PKA signaling is involved in the regulation of flavonoid biosynthesis in *H. androsaemum* cell cultures. **Acknowledgements:** *FCT for financial support (PEst-OE/SAU/UI0177/2011)* **References:** [1] Novais, M. *et al.* (2004) J. Ethnopharmacol. 93: 183 – 195 [2] Xavier, C. *et al.* (2012) Food Funct. 3: 844 – 852 [3] Hernandez, M. *et al.* (2010) Food Chem. 120:1076 – 1082 [4] Paranhos, A. (2006) Planta Med. 72: 1060 – 1061 [5] Paranhos, A. (2007) Planta Med. 73: 1017.

PN81 The physiochemical characteristics and anti-oxidant activity of extracts of *Platycodon grandiflorus* (Jacquin) A. De Candolle

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This study was performed to reduce unpleasant taste and flavor of *Platycodon grandiflorus* (Jacquin) A. De Candolleas well as to improve utilization as functional food materials using fermentation. We prepared 3 species of plant originated lactic ferment, the hot water extracts of dried *Platycodon grandiflorus* was incubated with lactic acid bacteria. These ferment products were evaluated viable cell count, pH, titratable acidity, organic acid, amino acid and DPPH radical scavenging activity during fermented 3 days. Viable cell count was increased in *Lactobacillus brevis* (LB) and *Leuconostoc mesenteroides* (LM) treatments. The pH and titratable acidity was decreased and increased, respectively. Total organic acid showed highly on LB treatment, especially more contained acetic acid contents. Total amino acid was highly on *L. plantarum* (LP) treatment, however, LB treatment was high in lysine contents of essential amino acid. DPPH radical scavenging activity showed higher activity on LB treatment for fermented 1 day and LP treatment was higher for

fermented 3 days. In sensory evaluations, LB treatment had as a best result of overall acceptance. Therefore, the extracts of fermented *Platycodon grandiflorum* with lactic acid bacteria which can help improve flavor through the process of fermentation and be applied as beverage resources in industrial area due to their effective flavor compounds and anti-oxidant activity.

PN82 Construction of a single-chain variable fragment antibody against daidzin and its potential use in an enzyme-linked immunosorbent assay

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Daidzin is one of the major isoflavonoids found in legumes. Several studies has reported estrogenic effects of this class of compounds. To develop an immunoassay to determine the concentration of daidzin in plant samples, a single-chain variable fragment antibody (scFv) against daidzin was constructed. The variable heavy and light chain genes were cloned from cDNA of hybridoma cell line. Then they were assembled together by splicing by overlap extension PCR (SOE-PCR) using specific primers designed to have flexible peptide $(Gly_{(4)}Ser)_{(3)}$ as linker. The constructed scFv gene was ligated into the pET28a expression vector and transformed into *E. coli* BL21 (DE3). The expressed scFv containing His6-tag at its N-termini was purified by immobilized metal ion affinity chromatography (IMAC). The purified scFv was characterized and applied to use in enzyme-linked immunosorbent assay (ELISA).

PN83 Glucose uptake stimulation of *Portulaca oleracea* extract in L6 myotube cells

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Portulaca oleracea (Portulacaceae) is a Thai local vegetable which its aerial part has been reported for its ability to decrease blood glucose *in vivo*. This study is aimed to investigate the glucose uptake stimulation effect of *P. oleracea* extract in L6 myotube cells. The dried aerial part of *P. oleracea* was macerated in 80% ethanol, and then it was concentrated and lyophilized to obtain crude extract (7.58% dry weight). The glucose uptake stimulation effect was determined by using 2-deoxy glucose assay. It was found that*P. oleracea* extract stimulated glucose uptake in dose-dependent manner with the highest level of 90% stimulation at the concentration of 300 μg/mL while the 500 nM insulin (positive control) showed 95% stimulation when incubated for 48 hrs without toxicity effect to cells. The increasing of glucose transporter mRNA (GLUT1 and GLUT4) level was obtained which could be inhibited by 3.5 μM cycloheximide. It can be concluded that *P. oleracea* extract exhibited glucose uptake stimulation into L6 myotubes via the up-regulation of GLUT1 and GLUT4 synthesis. These results suggest the possible promotion of *P. oleracea* as vegetable for reducing blood sugar level. .

PN84 The therapeutic role of *Sesbania grandiflora* as an inhibitor of Advanced Glycation Endproduct (AGE) formation and the discovery of lead compounds for managing hyperglycaemia

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The purpose of the study is to evaluate Advanced Glycation Endproduct (AGE) and early glycation (HbA_1c) inhibition by the greens of *Sesbania grandiflora*. The inhibition of the digestive enzymes (α-amylase and α-glucosidase) and the evaluation of antioxidant capability were also carried out. Put together, the aim of the study is to reveal the scientific mechanism behind the usage of *Sesbania grandiflora* in the management of *Diabetes mellitus.* AGE inhibition was performed by incubating haemoglobin with glucose for three weeks and characterized by fluorescence spectroscopy. Inhibition of glycation formation was done on human blood and characterized by micro-column ion exchange chromatography. The enzymatic inhibition of α-amylase and α-glucosidase was performed using starch and *p*-nitrophenyl-α-D-glucopyranoside as substrate. Antioxidant activity was evaluated by reducing power assay, nitric oxide scavenging and super oxide scavenging assay. Fatty acid profile and alkaloids were analysed using Gas Chromatography-Mass Spectrometry. The extract turned to be a better inhibitor of AGEs. The fluorescence signals produced by the formation of AGEs were reduced in the presence of plant extracts. Fluorescence was calculated after subtracting respective plant extracts as control. In the early glycation inhibition assay, glycated blood samples showed an increase of HbA_1c% from 3.95% to 9.56% in 22 h. Methanol extract of *Sesbania grandiflora* greens inhibited the formation of early glycation by 50% (HbA1c-4.69%). The methanol extract of *Sesbania grandiflora* showed significant inhibition of α-amylase (52%) and α-glucosidase (56%). The methanol extract contains derivatives of piperidine, cinnamaldehyde and linolenic acid which were proven anti-diabetic agents. From our results we conclude that further investigation of *Sesbania grandiflora* toward anti-diabetic drugs will be fruitful.

PN85 Seasonal variation of the content and composition of essential oils in *Betula* spp. leaves naturally growing in Estonia

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The specific terpenoids as potential chemosystematic markers for some birch species (*Betula* spp., Betulaceae) were found in our previous investigation. The aim of the current study was to compare the content and composition of essential oils in four *Betula* species naturally growing in Estonia. The leaves of *B. pendula, B. pubescens, B. humilis* and *B. nana* were collected in the middle of June, August and October from Tallinn, Risti bog and Viru bog in Estonia. The yields of essential oils were analysed by hydrodistillation and their chemical compositions were studied using GC and GC-MS. The yield of essential oil was the highest (0.11 – 0.27%) in the leaves of *B. humilis*, and not detectable in the leaves of *B. nana*. The leaves of the other birch species studied had low concentrations of oil: 0.11% in the *B. pendula* and 0.05% in the *B. pubescens* leaves collected in spring, the amounts of oils were not detectable in their leaves in August and October. *a*-betulenol (0.9 – 33.1%), *a*-betulenol acetate (2.9 – 31.9%), *b*-betulenal (2.5 – 7.6%) and *b*-betulenol (0.3 – 2.0 – 5.8%) were identified as the main constituents of leaves' oil of *B. pendula, B. pubescens* and *B. humilis*, respectively. The composition of the essential oil of *B. pubescens* resembled more to oil distilled from *B. humilis* than the oil from *B. pendula*. The variation of the main terpenoids in *B. nana* essential oil was rather different than in the other *Betula* species studied. A strong negative correlation ($r = -0.903$; $p < 0.01$) between the total content of aliphatic compounds and bicyclic sesquiterpenoids was found. The total amounts of bicyclic sesquiterpenoids (birkenal, δ-cadinene, τ-cadinol, a-betulenol, b-betulenol, b-betulenal, a-cadinol, 6-hydroxy-b-caryophyllene acetate, a-betulenol acetate) showed the tendency of decreasing, and aliphatic compounds (*n*-heptadecane, *n*-hexadecanal, *n*-nonadecane, palmitic acid, *n*-eicosane, *n*-octadecanal, *n*-heneicosane, *n*-tricosane, and *n*-pentacosane) increased from spring to autumn.

PN86 Mineral elements and phytochemical analysis of *Calendula officinalis* L. affected foliar application of Bio-stimulators

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Calendula officinalis L. (Asteraceae) is an important ornamental and medicinal plant. This experiment has been conducted on the base of randomized complete blocks design with three replications in 2011. The treatments included bio-stimulators with commercial formulations of Aminolforte, Kadostim, Fosnutren, Humiforte (each of them 0.75 and 1.5 $L.ha^{-1}$), composition of chemical fertilizer NPK (16 – 4-8), and control treatment (without foliar application). The results showed that the treatments had significant ($P < 0.01$, $P < 0.05$) effect on the studied parameters. The maximum amount of capitula dry weight (46 $g.m^{-2}$), leaves dry weight (25.2 $g.m^{-2}$), total dry weight (161.2 $g.m^{-2}$), tolal carbohydrates of capitula (2.282 $mg.g^{-1}$ DW) were observed with Humiforte 1.5 $L.ha^{-1}$. The highest amount of total carbohydrates in leaves (0.244 $mg.g^{-1}$ DW) was observed with Kadostim 1.5 $L.ha^{-1}$. The most

content of total flavonoids in leaves (0.1%) and capitula (0.25%) was obtained in Humiforte 1.5 L.ha^{-1} and Aminolforte 1.5 L.ha^{-1}, respectively. Content of macroelements (N, P, K) and microelements (Fe, Zn, Cu, Mn and Ca) increased with foliar application of bioactive amino acid compounds. In general, Humiforte and Kadostim 1.5 L.ha^{-1} were the best treatments in respect of morphological traits and Fosnutren, Aminolforte and Kadostim 1.5 L.ha^{-1} for phytochemical characters which can be due to existence of amino acid compounds and macro-nutrients such as Nitrogen, Phosphorous and Potassium in their formulations. **Key words:** *Calendula officinalis* L., bioactive amino acid compounds, Morphological and Phytochemical characters **References:** [1] Thomas J, Mandal AKA, Raj Kumar R, Chordia A. Role of biologically active amino acid formulations on quality and crop productivity of Tea (Camellia sp.). Int. J. Agric. Res. 2009; 4: 228-236.

PN87 Mode of action of a proanthocyanidin (PA)-enriched extract from Cranberry (*Vaccinium macrocarpon* Ait.) against uropathogenic *Escherichia coli* (UPEC): antiinvasion *versus* antiadhesion

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One of the most common infectious diseases are urinary tract infections (UTI) mainly caused by UPEC with rising antibiotic resistance. Therefore, the need for alternative therapies and prophylaxis is evident. Cranberry fruit extracts are traditionally used for prevention of recurrent UTI with moderate clinical evidence. The common opinion on the mode of action implies that A-type proanthocyanidins inhibit the adherence of UPEC to bladder epithelium by interacting with P-fimbriae and type 1 fimbriae. Our study aimed to investigate potential mechanisms of anti-infective properties of Cranberry extract. Antiadhesive activity of a PA-enriched, standardized Cranberry extract was investigated using fluorescence-labeled UPEC (strain 2980) and T24 human bladder epithelial cells with flow cytometric evaluation. Surprisingly samples containing Cranberry extract lead to much higher fluorescence intensity compared to the untreated control. This indicates increased bacterial adhesion to the host cells. This result is in total contrast to the common hypothesis that Cranberry inhibits bacterial adhesion to bladder cells. Confocal laser scanning microscopy was done to obtain multidimensional image data sets, which also demonstrated significantly increased adhesion of UPEC to host cells with typical agglutination of UPEC on the outer side of cell membranes at extract concentrations of 10 µg/mL. Clusters were exclusively located on the surface of the bladder cells, while the inside was almost completely free of UPEC. Also Scanning Electron Microscopy confirmed this finding and clearly indicated typical cluster formation on the epithelial outside.

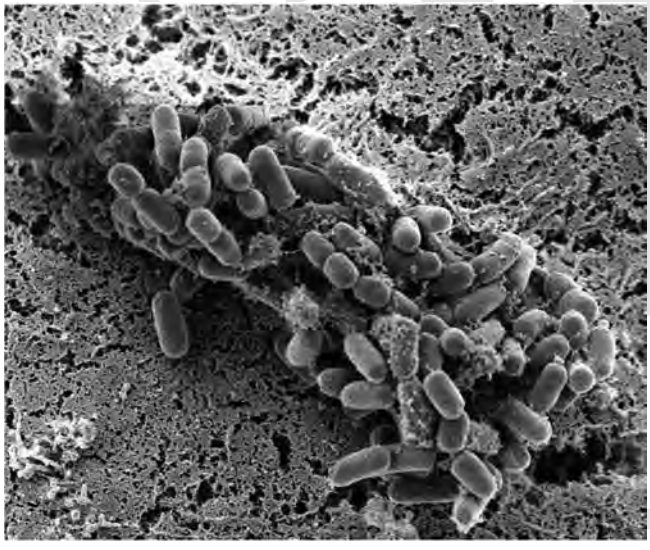

Fig. 1: Cluster of UPEC after treatment with Cranberry extract (25 µg/ml) and adhesion to T24 bladder cells. SEM 5000 x

An *in vitro* invasion assay revealed that UPEC-clusters, adhering to the cell surface, are not internalized anymore: the inhibition of bacterial invasion by the extract (50 µg/mL) was > 88%. Obviously it can be deduced that Cranberry extract exhibits an increase in bacterial adhesion to bladder cells, but inhibits the internalization into them.

PN88 Genotoxic and antigenotoxic activities of washed water extracts from brown jasmine 105 Thai rice cultivar in human lymphocytes *in vitro*

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The chemopreventive potential of rice extracts against various cancer, such as breast, liver and colorectal cancer, has been increasingly stated in many reports (1,2). Their mechanisms of action are still unclear. One possible mechanism might be their antigenotoxicity. This study aims to investigate the genotoxic and antigenotoxic activities of washed water extracts from brown jasmine 105 Thai rice (WWBJR) cultivar in human lymphocytes by comet assay *in vitro*. The washed water from rice is commonly used in Thai traditional medicine especially for anti-inflammation, aphthous ulcer and as aqueous adjuvant. Treatments of WWBJR extract (rice:water 1:2; yield 0.3 g/kg) at the concentrations of 0.7, 7, 70, 700, 7000 ng/µl alone with isolated human lymphocytes (40,000 cells/µl) for 5 min were performed for genotoxic study. For antigenotoxicity, lymphocytes were treated with WWBJR at the same concentrations for 5 min, washed, and then treated with doxorubicin (Drb; a genotoxic chemotherapeutic agent) (0.1 µg/ml) for 5 min. Isolated lymphocytes alone and PBS-treated lymphocytes were used as negative controls whereas Drb (0.1 µg/ml) and hydrogen peroxide (294 mM) were used as positive controls. The results indicated that the WWBJR at all concentration tested could protect cells from genotoxic damage induced by Drb while WWBJR alone did not induce DNA damage above that of the negative controls. Instead, WWBJR at 70 ng/µl or more could significantly decrease DNA damage below that of the control ($p < 0.05$). Therefore, these WWBJR extracts should be beneficial for chemoprevention. *In vivo* study of these extracts are needed for further investigation in detail. **Acknowledgements:** This study was supported by Research Fund, National Research Council of Thailand (NRCT) **References:** [1] Katayama M, Sugie S, Yoshimi N, Yamada Y, Sakata K, Qiao Z, et al. Oncology reports. 2003;10(4):875 – 80. [1] Hui C, Bin Y, Xiaoping Y, Long Y, Chunye C, Mantian M, et al. Nutrition and cancer. 2010;62(8):1128 – 36.

PN89 Induction of apoptosis in human lung cancer NCI-H226 cells by ethanolic extract of Benjakul preparation and its isolated compound

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Benjakul is a Thai traditional preparation used for lung cancer treatment. It is composed with five plants: *Piper chaba* fruit [PC], *Piper sarmentosum* root [PS], *Piper interruptum* stem [PI], *Plumbago indica* root [PL] and *Zingiber officinale* rhizome [ZO]. The previous report, plumbagin as a main component isolated from its ethanolic extract [1]. It also showed the highest cytotoxic against lung cancer cell but it has never been reported about its apoptosis mechanism with human non-small cell lung cancer NCI-H226 cells. Thus, plumbagin and its ethanolic extract were investigated cytotoxic activity against human non-small cell lung cancer NCI-H226 cells by using the sulforhodamine-B (SRB) assay [2], and their apoptosis mechanisms through cell cycle arrest and apoptosis [3]. The ethanolic extract of Benjakul and plumbagin exhibited high cytotoxic activity against NCI-H226 with IC_{50} values of 13.00 ± 0.72 µg/ml and 0.57 ± 0.16 µg/ml, respectively. Cell cycle analysis showed that both Benjakul (150 µg/ml) and plumbagin (1.5 µg/ml) induced similar G2/M phase arrest at 12 h and increased in apoptotic sub-G1 phase in a time-dependent manner. Similarly, annexin V FITC/PI analysis revealed

that the percentage of early apoptotic cells increased with the increasing treatment period of Benjakul extract and plumbagin ranging from 1.17% to 33.29% and 0.91% to 22.49%, respectively. The present study showed that Benjakul preparation and plumbagin induced cytotoxicity through cell cycle arrest at G2/M arrest and apoptosis in a time-dependent manner. All these findings provide information on the anticancer mechanisms of Benjakul preparation for the first time, thus these results support its promising traditional use for cancer treatment. **References:** [1] Sakpakdejaroen I and Itharat A 2009 J Health Res, 23(2), 71 – 76. [2] Skehan, P. et al,1990. J National Cancer Institute. 82:1107 – 1112. [3] Riccardi, C and Nicoletti, I (2006) Nature Protocols,1(3), 1458 – 1461.

PN90 Cholinesterase and tyrosinase inhibition studies and antioxidant activities of four *Onosma* species from Turkey

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The genus *Onosma* (Boraginaceae) is represented by 87 species in Turkey (1). The roots of some *Onosma* species are used for wound healing, burns, hemorrhoids, gastric ulcer, and tonsillitis in Turkey (2,3). The roots of some *Onosma* species are known to be rich in napthoquinones. In our previous study, some naphthoquinones namely deoxyshikonin, acetyl shikonin and β,β-dimethylacrylshikonin were purified by various chromatographic techniques from n-hexane-dichloromethane (1:1) extracts of the roots of *Onosma* species studied (4). In the present research, as a part of our studies on *Onosma* species, we aimed to investigate cholinesterase and tyrosinase inhibitory potential of the n-hexane-dichloromethane (1:1) extracts of the roots of *O. nigricaule* (endemic), *O. obtusifolium* (endemic), *O. tauricum* and *O. armeniacum*. Napthoquinones isolated were tested against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) as well as tyrosinase (TYRO) at 100 mg/mL using microtiter plate assays by ELISA. Since oxidative damage is associated with neurodegenerative diseases, the extracts were tested for their antioxidant effect by five in vitro assays including 2,2-diphenyl-1-picrylhydrazyl, *N,N*-dimethyl-*p*-phenylendiamine, and nitric oxide radical quenching effect, metal-chelation effect, and ferric-, and phosphomolibdenum-reducing antioxidant power. According to the results, only the extract of *O. nigricaule* exerted a notable inhibition against AChE, against BChE, and the highest tyrosinase inhibitory activity as well. Acetyl shikonin and β,β-dimethylacrylshikonin also possessed moderate BChE inhibition and phosphomolibdenum-reducing antioxidant power. **References:** [1] Riedl H. (1978) *Onosma* L. in "Flora of Turkey and the East Aegean Islands" Vol. 6, pp. 326 – 376. Edinburgh Uni. Press, UK. [2] Ozgen U. et. al. (2006) J. Ethnopharmacol. 104, 100 – 103 [3] Cadirci E. et. al. (2007) Chem. Biol. Interact. 170(1), 40 – 48 [4] Ozgen U. et. al. (2010) Planta Medica 76, 1232 – 1232.

PN91 Effect of nitrogen and compost different levels on qualitative and quantitative performance (flower dry weight, seed dry weight, flower diameter, number of flower in plant, flavonoid content) of *Calendula officinalis* L.

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In order to investigate the effects of nitrogen and compost different levels on qualitative and quantitative performance of *Calendula officinalis L.* herb, an experiment was carried out in the research field of Chalous Azad University in 2011 – 2012. The experiment was done in factorial form as a randomized complete block design, in three replicates. Treatments consisted of nitrogen and compost. Considered nitrogen levels consisted of N_0= 0, N_1= 50, N_2= 100 kg/ha and compost levels were including C_0= 0, C_1= 6, C_2= 12 ton/ha. Investigated characteristics consisted of flower dry weight, seed dry weight, number of flowers in plant, flower diameter, flavonoid content. The results showed, nitrogen and compost treatments had statistically significant influence ($p \leq 0.01$) on studied characteristics. Flower dry weight, seed dry weight, flower diameter and number of flower in plant characteristics has been studied in eight harvest; as, the performance of these characteristics had increasing procedure from the first harvest up to the forth harvest; and, in the forth harvest, it has reached to its' maximum level and from fifth harvest, it had decreasing procedure. As, up to the forth harvest, the maximum flower dry weight, seed dry weight, flower diameter and number of flower in plant obtained by $C_{1\,x}\ N_2$ (C_1= 6 ton/ha compost and N_2= 100 kg/ha nitrogen) treatment and from fifth up to the eighth harvest, it was obtained by $C_{2\,x}\ N_2$ (C_2= 12 ton/ha compost and N_2= 100 kg/ha nitrogen) treatment. Also, the maximum flavonoid content obtained by $C_{2\,x}N_1$ (C_2= 12 ton/ha compost N_1= 50 kg/ha nitrogen) treatment. In conclusion, application of compost as a biological fertilizer plays an effective role in enhancement of quantitative performance and increment of the flavonoid content of the plant

PN92 Phytochemical and pharmacological screening of extracts from *Gunnera tinctoria* Mol., a native chilean plant

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Gunnera tinctoria Mol.((Gunneraceae), is a native plant from Chile. In spite of the traditional use of aqueous extracts from petiols and leaves of this species to treat inflammation, no scientific studies are available, and the potential use of its major components as a dermo pharmaceutical anti-inflammatory ingredient has not been explored thus far.[1] This study aimed to corroborate their presumed antiinflammatory activity, identify their active ingredients and validate their traditional use. A phytochemical and pharmacological screening was performed to determine the presence of secondary metabolites. The determination of total polyphenols was measured spectrophotometrically using the Folin-Ciocalteu method. All samples, were subjected to topical assays for the inhibition of inflammation elicited by arachidonic acid (AA) or phorbol ester (TPA), inducing inflammation on the mice ear.[2] The presence of secondary metabolites in the phytochemical screening was detected principally in aqueous, methanolic and ethyl acetate extracts. All extracts showed anti-inflammatory effect. Ethyl acetate extract had already been shown to exhibit strong anti-inflammatory effects in both models AA and TPA, 55.0% and 54.7%, respectively. **References:** [1] Estomba D., Ladio A., Lozada M. Medicinal wild plant knowledge and gathering patterns in a Mapuche community from North-western Patagonia. *J. of Ethnopharmacol.* **2006**, 103: 109 – 119 [2] Rodríguez-Díaz M, Delporte C, Cassels BK, González P, Silva X, León F, and Wessjohann L. Topical anti-inflammatory activity of quillaic acid from *Quillajasaponaria* Mol. and some derivatives *J Pharm Pharmacol* **2011**; 63: 718 – 724.

PN93 Anatomical characteristic and comparison enter the contents total alkaloids of *Peganum harmala* (Zygophyllaceae) the high plateaus of the area of Djelfa (Algeria)

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The purpose of our work is to know the Algerian natural resources in producing plants of alkaloids indolic and improve the production of alkaloids in Algeria considering the interest which these products represent, while being interested in the extracts of a species of the family of Zygophyllaceae: *Peganum harmala*. The objective of this study is the evaluation of the productive capacity of the total alkaloid for their eventual industrial exploitation. The results show a significant variability of the contents total alkaloids between organs collected at the various periods of the year and according to the environmental factors, in particular the climatic characteristics. The majority of the harvested June organs contain a high content of alkaloids. We have the example of the roots harvested June, contain 3,006% of Alkaloids Totals. While those collected the month of April contain 1,128% AT. The largest content was revealed in the seed collected in June with 4,269% AT. According Hammiche and Merad (1997), the alkaloid content rises sharply in the summer, during the period of fruit ripening, when the harvest of the seed. The anatomical study of *Peganum harmala* revealed the presences of the spots which we suppose of alkaloids are stored everywhere in the or-

gans, but they are more abundant in the collenchyma stems. **Key words:** Zygophyllaceae – *Peganum harmala* – total alkaloids – high plateaus – anatomy. **References:** [1] Hammiche, V. and Merad, R., (1997). *Peganum harmala L. (PIM 402F, French)* (em french). International Programme on Chemical Safety. Página visitada em 2008.

PN94 Antimicrobial, anticancer and anti HIV-1 of *Citrus volkameriana*

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Genus *Citrus* was well known as it was used in traditional medicine to cure many types of diseases from minor aliment to serious diseases (Arias & Ramon-Laca,2005). Recent studies proved that *Citrus* extracts exerted many activities such as anticancer, anti-inflammatory, antioxidant (Hamdan et al., 2011), analgesic, antibacterial, antifungal (Cvetnic & Vladimir-Knezevic,2004). The antimicrobial activity of *Citrus volkamariana* using p-iodonitrotetrazolim violet (INT) assay was used to determine the minimum inhibitory concentration (MIC). Petroleum ether fraction, dichloro methane fraction and volatile oil of the leaves showed antimicrobial activity against gram positive bacteria *Micrococcus luteus* B-287 at concentration (31.25, 62.5, 31.25 ug/ml). Aquoues fraction, apigenin and volatile oil showed anti *Mycobacterium tuberculosis* at the same concentration (125ug/ml). The anticancer activity of the plant using growth inhibitory effects were tested in the 3-cell line panel consisting of TK10 (renal), UACC62 (melanoma) and MCF7 (breast) cancer cells using a Sulforhodamine B (SRB) assay (Lopez-lazaro M et al., 2003) Petroleum ether fraction can be considered active for UACC-62 melanoma cell line (-50.5%) and the Volatile oil (-62.91%) which demonstrate selectivity at the breast cancer cell line MCF-7. Apigenin also showed antimelanoma activity against UACC-62(-77.5%) at concentration of 100 ug/ml. Petrolium ether fraction showed anti-HIV-1 activity (100 ± 0.00) at concentration (40ug/ml) measured by the syncytia formation assay (Reed and Muench, 1938). **References:** [1] Arias and Ramon-laca, 2005. Journal of Ethnopharmacology 97 (2005) 89 – 95. [2] *Hamdan et al., 2011. Virology Journal* 2011, 8:217. [3] Cvetric and Vladimir-Knezevic, 2004. Food Chem Toxicol., 40:20, 1731 – 43. [4] Lopez-LazaroM. et al., 2003. Planta Med. 2003 Aug;69(8):701 – 4. [5] Reed and Muench, 1938. The American Journal of Hygiene 27: 493 – 497.

PN95 Tablet Formulation and Stability Test of Thai Traditional Remedy for Muscle Pain Treatment called Sahasthara

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A Thai Traditional remedy called Sahasthara (SHT) is normally used to treat muscles pain and arthritis in the National drug list of herbal medicinal products A.D. 2011 of Thailand. Its formula consists of twenty one plants. Its ethanolic extract of SHT exhibited anti-inflammatory activity via inhibition of NO production with IC_{50} as 2.06 µg/ml. Piperine was isolated from its extract and also tested by inhibitory effect on NO production. It showed higher anti-inflammatory than indomethacin as positive control (IC_{50} as 11.48 and 20.32 µg/ml). The tablet form of the ethanolic extract of SHT remedy was developed and tested product stability under accelerated condition. Preformulation of SHT extract was investigated under various storage conditions; moisture, acid-base, temperatures and oxidation environments. The results exhibited that SHT extract was stable in base, high temperature but unstable in acid and oxidation environments. A direct compression method was used in developing the tablets. The suitable excipients were microcrystalline cellulose (Avicel® PH 102), croscarmellose sodium (Ac-Di-Sol®), Hydrophilic fumed silica (Aerosil®) and magnesium stearate. The physical properties of tablets were evaluated and accepted following by the USP25 requirements. The results of stability test of its tablet was investigated by using HPLC and found that piperine as a main compound was remained as 88.94% at day 120. These results concluded that its tablet was stable and shelf life more than 2 years.

PN96 Enhancement of anthraquinone production in *Cassia tora* root cultures using medium manipulation technique

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Anthraquinone (AQ) such as aloe-emodin, chrysophanol, emodin and physcion are valued pharmacological active compounds found in *Cassia tora*. Root cultures of *C. tora* have been established in order to study on their AQ production. *C. tora* root cultures were established from root explants in Murashige & Skoog (MS) medium supplemented with 0.1 mg/L α-naphthaleneacetic acid (NAA) and 1.0 mg/L kinetin (Kn). In this study effects of basal media and plant growth regulators on growth and AQ production of *C. tora* root cultures were evaluated. Variety of basal media including Gamborg's B5 (B5), MS and Nitsch & Nitsch (NN) supplemented with 0.1 mg/L α-naphthaleneacetic acid (NAA) and 1.0 mg/L kinetin (Kn) were used. The 4 week-old root cultures were harvested. The biomasses were recorded and the harvested roots were subjected to HPLC quantitative analysis of AQ. Biomass of the root cultures reached the highest when cultured in B5 medium (0.44 ± 0.045 g DW/flask) while NN (0.25 ± 0.078 g DW/flask) and MS (0.19 ± 0.065 g DW/flask) were not appropriate for growth of the root cultures. AQ production in the root cultures from MS and B5 media were comparable (2.35 ± 0.142 mg/g DW and 2.31 ± 0.133 mg/g DW, respectively). Considering the growth and the AQ production, B5 was the most appropriate media for culturing *C. tora* root cultures. Optimal level of Kn and NAA in B5 medium were further investigated. Kn greatly influenced on AQ production in dose dependent manner whereas NAA affected on root morphology and biomass. Optimization of Kn and NAA concentrations revealed that B5 medium supplemented with 1.0 mg/L NAA and 2.0 mg/L Kn gave the highest content of AQ in the root cultures (3.59 ± 0.166 mg/g DW) which was 1.6-fold fold higher than the levels of the non-optimized group.

PN97 Effectiveness of bulb extracts of *Allium* species on some selected plant pathogenic fungi

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The antimicrobial activity of *Allium* species has been recognized since a long time, with allicin, further thiosulphinates, and their transformation products having antimicrobial activity. As a last aspect of all investigations, the sulphur chemistry of *Allium* species located in South West and Middle Asia is much more complex and diverse than the chemistry of those species which were traditionally used in the Western World. *Allium* volatile compounds of the species from Asia seem to be an excellent source for new sulphur compounds and aroma constituents. The purpose of this study was to determine and compare the antifungal activity (Minimum Inhibitory Concentration) of *Allium sativum* L., *A. cepa* L., *A. stipitatum* Regel, *A. atroviolaceum* Boiss. and miconazole (positive control) on *Aspergillus flavus*, *A. niger*, *Penicillium digitatum*, *P. italicum* and *Mucor hiemalis*, using broth micro-dilution susceptibility testing method, disk diffusion method, PDA micro-dilution susceptibility testing method and double-dish chamber. Different concentrations of *Allium* extraction using ethyl acetate dissolved in DMSO were tested on all the above mentioned fungi, thus *A. sativum* (garlic) showed the highest antimicrobial effect against all the tested fungi (MIC ≥0.3gr/ml; related to dried total extract) followed by *A. cepa* (onion) and *A. stipitatum* (MIC ≥1gr/ml) and then *A. atroviolaceum* (MIC ≥2.5gr/ml). The MIC of miconazole as a control was ≥0.04mgr/ml. From the fungal point of view, *P. italicum* showed the highest susceptibility, while *M. hiemalis* and *A. flavus* demonstrated more resistancy towards *Allium* extracts and Miconazole. Meanwhile *A. sativum* inhibited *A. niger* more than Miconazole, while having only little effect on *P. digitatum*. The results indicate that extractions of *Allium* spp. have antifungal activity and might be promising, at least, in 'biological' treatment of fungal-associated plant diseases.

PN98 Appropriate extraction method for high contents of total phenolics, total flavonoids and free radical scavenging properties of *Ziziphus mauritiana* seed extracts

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Ziziphus is a genus in the family Rhamnaceae which contains about 40 species. Fruits and seeds of *Ziziphus* have been widely used as traditional medicines since ancient time. *Z. mauritiana* is a tropical or subtropical fruit tree commonly distributes in many Asian countries. The seeds of *Z. mauritiana* have been reported as anticancer, antidiabetic and also used to treat insomnia, reduce the body temperature and sweat. Four different extraction methods, i.e. maceration, decoction, soxhlet extraction and sonication were carried out for extracting dried powder of *Z. mauritiana* seeds. The solvent used for maceration, sonication and soxhlet extraction was 50% and 80% ethanol, while distilled water was used for decoction. All extracts were investigated for the yields of crude extract, free radical scavenging activity using DPPH assay, ferric reducing antioxidant power by FRAP assay, the contents of total flavonoids and total phenolics by colorimetric assay and Folin-Ciocalteau assay, respectively. Comparing between *Z. mauritiana* seed extracts from various extraction methods, sonication procedure provided the extracts with high total flavonoids content. However, the extract from 50% ethanol using soxhlet extraction with low temperature (60°C) exhibited good DPPH scavenging activity with EC_{50} of 52.91 ± 1.00 µg/mL with high total phenolics content of 27.62 ± 1.43 mg gallic acid equivalent (GAE)/g extract. The results suggested that the polyphenolic contents had high relation to antioxidant activity. Therefore, soxhlet extraction with 50% ethanol should be recommended as the appropriate extraction method for *Z. mauritiana* seeds for further pharmaceutical development.

PN99 The Influence of Toll-like Receptor (TLR-) Agonists on Lysozyme Activity and TNFα-Secretion in THP-1 cells

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TLRs (Toll-like receptors) are part of the innate immune system and play a major role in the recognition of pathogens. They participate in the stimulation of monocytes and macrophages by activation of NF-κB and C/EBPβ, two transcription factors that are implicated in lysozyme and TNF-alpha expression. Aim of this study was to investigate the influence of TLR-agonists and other natural compounds on cytokine secretion and lysozyme activity in a comparative assay. To explore the importance of differentiation THP-1 cells were pre-treated with PMA and IFNy. Stimulation of undifferentiated cells with natural products including various bacterial antigens, diterpenes and alkamides had almost no effect on TNF-alpha secretion and lysozyme activity whereas secretion of IL-6 and IL-10 as well as intercellular adhesion was clearly affected. Differentiated THP-1 cells were much more sensitive to further stimulation yet revealed an opposing effect on lysozyme activity and TNF-alpha secretion. The results suggest a coordinated activation of intercellular adhesion and cytokine production in the differentiation process from monocytes to macrophages and highlights the role of natural products as putative immunmodulatory agents.

PN100 Microalgae: From culture medium to medicine

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Microalgae have become an important commercial source of many valuable compounds, such as carotenoids, lipids, proteins, polysaccharides, used in pharmaceutical and industrial purposes. As a result of this rich composition, microalgae-derived products consumed by people for many reasons; for health improvement, for cosmetic use and as food supplements. Also, they have been used as feed ingredient for animals. *Spirulina platensis, Dunaliella salina, Haematococcus pluvialis and Chlorella sp.* are very well-known microalgae species which are used in pharmacologic field all around the world. In general, microalgae have hepatoprotective, antimicrobial, antioxidant, immunstimulant activities; and also they have colouring properties as natural dyes for medicines, foods, cosmetics and analitical applications (1). In the present study, microalgae samples were collected from different geothermal water supplies in Afyonkarahisar, Turkey. At the first glance, cyanobacteria, diatoms and some coccus microalgae strains were defined. These strains were isolated as a single strain and cultured in their own geothermal water, at 30 ± 2 °C, 24 h light period of 20 µmol m^{-2} s^{-1}. Biomass concentration was determined with spectrophotometric method, and simultaneously controlled chlorophyll-α and dry mass analysis. An HPLC method was developed to discern the variety and content of microalgae carotenoids. Some HPLC standarts, beta-carotene, astaxanthin and canthaxanthin were purchased and lutein, violaxanthin and neoxanthin were isolated from spinach (2). DPPH (1,1 – diphenyl-2-picrylhydrazyl), SO (superoxide) and NO (nitric oxide) radical scavenging activity methods were used to highlight the antioxidant potential of the samples. In conclusion, some microalgae can also be cultured in geothermal water as a source of bioactive compounds for medicinal and nutritional use. **References:** [1] Mendes, R. et al. (2003) Inorg Chim Acta, 356, 328. [2] Kao, T.H. et al. (2012) J Pharm Biomed Anal, 66, 144.

PN101 Comparative chemical composition, essential oils and antibacterial activities of *Pinus halepensis* Mill. North- East from Algeria

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In Algeria *P. halepensis* Mill. forms the forest in the dry party for Mediterranean littoral and the hills and mountains semi-arid on-road and extends up to 2200 m in the internal dry mountains and the calcareous soils coast. The essential oil of the needles of *Pinus halepensis* Mill., collected in the lake Mellah forest in the National Park of El Kala (P.N.E.K) and the forest of Zaarouria (Souk Ahras) was also obtained by hydrodistillation. The yield is respectively 0.81%, 0.3%. The essential oil of *Pinus halepensis* Mill. lake Mellah is composed mainly of: β-caryophyllène (31, 89%), α- pinène (24, 41%), Δ3-carene (19,38%), While that of Zarouria consists primarily of β-caryophyllène (32,3%), α-pinène (21,79%), α -terpinolène (9,78%). The antibacterial activities of essential oils were tested against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginos*. The tested essential oilsshowed antibacterial activity against bacterial pathogens used, in particular against *Staphylococcus.* **Keywords:** Essential oils, *Pinus halepensis* Mill., antibacterial activity, CG/SM.

PN102 A novel chemical synthesis of biflavonoid Rhodophyscin

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Rhodophyscin is a biflavanoid ubiquitously occuring in plants, and recently we isolated it from trees of *Eucommia*, *Paulownia*, and *Juglans*. It has been reported that rhodophyscin exhibit various bioactivities including antiinflammatory, antioxidant, neuroprotective, antitumor, antibacterial and antiviral properties [1]. We introduced an efficient synthetic method to achieve rhodophyscin in large scale. Briefly, *p*-anisaldehyde was iodinated at the 3-position to give 3'-iodo-4-methoxybenzaldehyde (1). Compound **1** was condensed with 2-hydroxy-4,6-dimethoxyacetophenone to forward 4,4',6'-trimethoxy-3-iodo-2'-hydroxychalcone (2). Compound **2** underwent a ring cyclization reaction to produce 4',5,7-trimethoxy-3'-iodoflavone (3). The iodine moiety in **3** was converted to the pinacol functionality to form 4',5,7-trimethoxy-3'-pinacolatoboronflavone (4). Meanwhile, 2',4',6'-trihydroxy-acetophenone (5) was first methylated at the 2 and 4 position to produce 2-hydroxy-4,6-dimethoxyacetophenone (6). Compound **6** was iodinated to forward 2'-hydroxy-3'-iodo-4',6'-dimethoxy-acetophenone (7) which was condensed with *p*-anisaldehyde to give 4,4',6'-trimethoxy-3'-iodo-

2'-hydroxy-chalcone (**8**). Compound **8** undertook a ring closure reaction to generate 4',5,7-trimethoxy-8-iodoflavone (**9**). A Suzuki reaction was applied by coupling **4** and **9** to achieve 4',5,7,4''',5'',7''-hexamethoxy-rhodophyscin (**10**). Finally **10** was subject to demethylation to obtain rhodophyscin. The novel synthesis method can be applied to produce other C-C biflavonoids and biphenyls. **References:** [1] Lin RC. et al. (1994) Planta Med 60: 168 – 170. **Acknowledgements:** This work was supported by National Natural Science Foundation of China (31170541, 31000279), Program for New Century Excellent Talents in University (NCET-10 – 0951), Natural Science Foundation of Tianjin City (13JCZDJC), and Foundation (200301) of Jiangsu Provincial Key Laboratory of Pulp and Paper Science and Technology, Nanjing Forestry University, China.

PN103 Antioxidative and neuroprotective activities of isocampneoside II on H_2O_2-induced oxidative injury in PC12 cells

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Oxidative stress has been considered as a major cause of cell damage in a variety of neurodegenerative disorders. One of the reasonable strategies for delaying the disease's progression is to prevent the reactive oxygen species (ROS) mediated cellular injury by dietary or pharmaceutical augmentation of free radical scavengers [1]. Isocampneoside II (ICD) is an active phenylethanoid glycoside compound isolated from *Paulownia tomentosa* var. *tomentosa* (Scrophulariaceae) [2]. The present study was designed to explore the free radical scavenging potential of ICD in different *in vitro* systems and its protective role in H_2O_2 induced oxidative stress and apoptotic death in cultured rat pheochromocytoma (PC12) cells. The results showed that ICD eliminated approximately 80.75% superoxide radical at the concentration of 0.1 mg/ml and inhibited metal chelating by 22.07% at 8 mg/ml. In addition, ICD showed a strong ability on reducing power and provided protection against oxidative protein damage induced by hydroxyl radicals. Pretreatment of PC12 cells with ICD prior to H_2O_2 exposure elevated the cell viability, enhanced activity of superoxide dismutase (SOD) and catalase, and decreased the levels of malondialdehyde (MDA) and intracellular ROS. Furthermore, ICD inhibited cell apoptosis and Bax/Bcl-2 ratio induced by H_2O_2. These findings suggested that ICD may be considered as a potential antioxidant agent and should encourage for further research in neurodegenerative diseases. **References:** [1] Hsu CL, et al. (2010). J Agric Food Chem 58: 2150 – 2156 [2] Si CL. et al. (2011) Holzforschung 62: 197 – 200. **Acknowledgements:** This work was financed by National Natural Science Foundation of China (31170541 & 31000279), Program for New Century Excellent Talents in University (NCET-10 – 0951), Natural Science Foundation of Tianjin City (No. 13JCZDJC), and Foundation (201204) of Tianjin Key Lab of Marine Resources & Chemistry, Tianjin University of Science & Technology, China.

PN104 Willow bark extract STW 33-I is safe and effective in the long term-treatment of outpatients with rheumatic pain esp. osteoarthritis or back pain – a subgroup analysis

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Objectives: Degenerative musculoskeletal diseases are of increasing relevance in an ageing population. The efficacy and safety of willow bark extract in the therapy of painful musculoskeletal disorders has been shown in a large number of clinical studies. This 6 months pragmatic surveillance study with STW 33-I (Proaktiv®, extraction solvent water; drug-extract ratio 16 – 23:1) included 436 patients with rheumatic pain mainly due to osteoarthritis and back pain. The patients were treated with STW 33-I monotherapy or in combination with NSAIDs and opioids. **Method:** An extensive case report form including pain questionnaires and patient diary was used for outcome evaluation. **Results:** All patients started treatment with STW 33-I. 61.5% of the patients (N = 268) took no analgetic comedication. 28.9% (N = 126) took a dual therapy with NSAIDs (mostly diclofenac or ibuprofen) and only 3.9% received a triple therapy (STW 33-I + NSAID + opioid). Only 5.7% (N = 25) used other analgesics like gabapentin. The mean change from baseline of the pain intensity score (VAS) was -22.4 (baseline 58,7) in the subgroup of the patients receiving STW 33-I only, -18.0 (baseline 56.0) in the STW 33-I and NSAIDs subgroup and -20.5 (baseline 67.4) in the STW 33-I, NSAIDs and opioids subgroup. These results were comparable to other scales (Likert-Scales, patients' diary) concerning pain intensity at rest and in motion and pain duration. The tolerability of STW 33-I was distinctly better compared to the other investigated subgroups. No relevant drug interactions were reported. **Conclusions:** These data suggest that the phytomedicinal approach, and especially STW 33-I (Proaktiv®), can be used as a basic treatment in the long term therapy of degenerative joint diseases and that it can be combined with NSAIDs and opioids if necessary.

PN105 *Arnica montana* L. cell suspension culture as a biotechnological approach for the production of bioactive secondary metabolites

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Arnica montana L. is a medicinal plant with traditional use to treat sprains, bruises, rheumatic and muscular aches based on the anti-inflammatory properties of sesquiterpene lactones (SL), mainly esters of helenalin and dihydrohelenalin. Overexploitation has led to conservation actions in the majority of European countries, involving *in vitro* cultivation techniques as a sustainable alternative. Cell suspension cultures of *A. montana* have not been reported extensively [1,2,3], although a sustainable biotechnological process in bioreactors for production of the active plant secondary metabolites is of great interest for industry [4]. The starting plant material consisted of seeds, collected from wild populations in the Romanian Eastern Carpathians. Germination in aseptic environment lead to sterile plantlets for the micropropagation process. Leaf fragments of about 1 cm^2 were placed on several medium variants – MS medium supplemented with different amount of *2*,4-dichlorophenoxyacetic acid and benzylaminopurine for callus induction. First qualitative results show that suspension cultures from the obtained calli in small scale single-use bioreactors have been successfully established. Due to small biomass amounts, common HPLC and HPTLC methods from the Ph Eur Monograph on *Arnica* for SL, flavonoids and phenolic acids using HPLC and HPTLC had to be optimized to very low sample amounts. Quantitative assessment of the desired secondary metabolites is under way in parallel to the continuous culturing process. The final aim of the study is to show differences in secondary metabolite content in plant material from the field, callus and cell suspensions. **Acknowledgements:** Sciex-HMS^{ch} Program CRUS Switzerland, No.11.273 **References:** [1] Schmidt TJ et al. (1998), Planta medica 64, 268 – 70 [2] Petrova M et al.(2012), Proc. of the 7th CMAPSEC, Serbia, 345 – 50 [3] Puhlmann J et al.(1991), Phytochemistry, 30 (4), 1141 – 5 [4] Petrova M et al. (2012), Acta Physiol Plant, 34, 1597 – 606

PN106 Antioxidant activity and glucose transport effect in 3T3-L1 and L6 cells of *Nelumbo nucifera* root extract

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Rhizome of *Nelumbo nucifera* Gaerth was long been used by Thai traditional doctors for treatment diabetic patients. The objective of this study

was to investigate the *in vitro* antioxidant activity and glucose transporter effect in adiposite cells (3T3-L1) and muscle cells (L6) of its ethanolic extract. The DPPH and inhibition of lipid peroxidation assay were used for determination of antioxidant activities [1,2]. The glucose uptake of radioactive 2-deoxyglucose in L6 and 3T3-L1 cells were used to measure the glucose transporter in both types of cells[3]. The EC_{50} of antioxidant activity by DPPH assay and inhibition of lipid peroxidation on liposome assay of its extract were 4.89 and 5.18 µg/ml respectively. The glucose transporter activity of *Nelumbo nucifera* extract in both L6 and 3T3-L1 showed glucose uptake increasing level by fold of basal. The ethanolic extract at concentration 0.1 µg/ml exhibited the highest glucose uptake level by 1.47 fold in L6-muscle and 1.69 fold in 3T3-L1 adiposite cells respectively. This result can support using the ethanolic extract of *Nelumbo nucifera* as antioxidant and glucose transporter drug on glucose uptake in both muscle and adipose cells. The further isolation of antioxidant and glucose transporter compounds from this plant should be researched and antihyperglycemic activity in animal model should be investigated. **References:** [1] Uchiyama M and Mihara M. (1978). *Analytical Biochemistry.* 86: 271 – 8. [2] Yamasaki K et al.,(1994). *Chem. Pharm. Bull.* 42:1663 – 5. [3] Nicky K. and Juan C, (2009) *Methods in molecular biology* 560: 111 – 35

PN107 Evaluation of anti-inflammatory and anti-cancer activities of *Lycopus uniflorus* fractions

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Lycopus uniflorus, referred to as bugleweed, is widely spread in Lebanon near freshwater wetland. The plant has been used in Lebanese folk medicine for the treatment of inflammation and gastrointestinal disorders. The present study aims to evaluate the potential effects of the methanolic extract of *L. uniflorus* leaves in acute and chronic inflammatory models using the carrageenan and formalin- induced rat paw edema models (1). We also investigated the anti-proliferative effects of petroleum ether, chloroform, ethyl acetate and n-butanol fractions on MDA (breast) and HT-29 (colon) cancer cells. The results showed that intraperitoneal pretreatment with doses of 200, 100 and 50 mg/kg BW caused 71%, 30% and 18% inhibition of acute inflammation respectively. Similarly, daily treatment over six days with the same doses exhibited 77%, 65% and 28% inhibition of chronic inflammation induced by formalin. Treatment with diclofenac (10 mg/kg BW) caused 75% and 61% inhibition of acute and chronic inflammation respectively. No signs of toxicity were shown at the administered doses. On the other hand, the results on MDA and HT-29 cells proliferation were more notable by ethyl acetate and chloroform fractions. The effects were dose-dependent for the ethyl acetate fraction on MDA (89%, 54% and 37%) and HT-29 cells (79%, 53% and 11%) at 200, 100 and 50 µg/ml, respectively. Similar effects were observed at the same doses for the chloroform fraction on MDA cells 82%, 49.5% and 28.5%) and HT-29 cells (65%, 40.5% and 15%), respectively. In conclusion, *Lycopus uniflorus* can be considered a potential source for anti-inflammatory and anti-cancer agents. **References:** [1] Chau, T.T., 1989. Analgesic Testing In Animal Models. In: Pharmacological Methods in the Control of Inflammation, Chang, J.Y. and A.J. Lewis (Ed.). Alan R. Liss, Inc., New York, pp: 448. *Acknowledgments:* Mr. Jean Karam.

PN108 *Salvia officinalis* for menopausal hot flushes: Towards determination of mechanism of activity and active principles

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Herbal medicinal products are commonly used in alternative treatment of menopausal hot flushes. In a recent clinical study, *Salvia officinalis* tincture was found to reduce hot flush frequency and intensity (1). This study aimed at investigating the mechanism(s) responsible for the anti-hot flush activity of *S. officinalis* and determination of its active principle(s). The ethanolic tincture and its *n*-hexane, $CHCl_3$ and aqueous EtOH subextracts were studied for *in vitro* estrogenic, selective serotonin reuptake inhibition (SSRI) and acetylcholinesterase inhibition (AChEI) activities. No activity was observed in the SSRI or the AChEI assays at the highest test concentrations, whereas the aqueous EtOH subextract displayed estrogenicity in the ERLUX assay (EC_{50} 64 µg/ml). Estrogenic activity-guided fractionation of this subextract identified luteolin-7-*O*-glucuronide (EC_{50} 129 µg/ml) as the active component of the initial VLC fraction 4 (EC_{50} 69 µg/ml). The most potent estrogenicity was tracked to a subfraction (7.6.7.6, EC_{50} 0.7 µg/ml) obtained from the VLC fraction 7 (EC_{50} 3 µg/ml). As this minor fraction (1.81 mg) could not be further purified, its constituents were characterised and quantified by both capillary NMR and UHPLC-PDA-TOF-MS to reveal luteolin-7-*O*-glucoside to be its major component. When the estrogenic activity of commercial luteolin-7-*O*-glucoside (EC_{50} 0.44 µg/ml) was compared to that of the subfraction 7.6.7.6, they were almost identical. Hence, luteolin-7-*O*-glucoside appears to contribute significantly to the estrogenicity of the fraction. This study suggests the involvement of ubiquitous estrogenic flavonoids in the *in vitro* anti-hot flush effect of *S. officinalis*, a safe and commonly used herbal medicinal product during the menopause. **Acknowledgements:** This project was financially supported by Bioforce AG, Switzerland. **References:** [1] Bommer S, Klein P, Suter A. *Adv Ther* 2011, 6, 490 – 500.

PN109 Genotoxicity and interference with cell cycle activities by an ethanolic extract from Thai *Plumbago indica* roots in human lymphocytes *in vitro*

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In Thai traditional medicine, *Plumbago indica* has been known to have health benefit especially for anti-inflammatory, antibacterial, and anti-tumor activities. However, the mechanisms of its action are still uncertain. One of which might be its genotoxic effect. In the present study, we investigated the genotoxicity of an ethanolic extract of *Plumbago indica* root (EEPIR) by sister chromatid exchange (SCE) assay in human lymphocytes *in vitro*. Dried EEPIR was prepared from percolation with 95% ethanol. Human lymphocytes were treated with EEPIR at concentrations of 12.5, 25, 50, 100 and 500 µg/ml in plain RPMI 1640 medium for 3 h at 37°C. The result indicated that EEPIR at the concentration of 12.5 – 100 µg/ml could induce cell cycle delay as shown by the significant increase in the number of metaphase cells in the first cell cycle but neither in the later cell cycles. Genotoxicity was found at 25 – 100 µg/ml EEPIR. Cytotoxicity was found at concentrations of > 500 µg/ml. Therefore, these activities of the EEPIR could serve its potential thera-

peutic effects, especially as anticancer. Further study of EEPIR *in vivo* is still needed to support this evidence.

PN110 Breeding of wild marjoram (*Origanum majorana* L.) for essential oil production

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Marjoram (*Origanum majorana* L.) is one of the important wild oregano species in Turkey. The most important characteristic of the species is high essential oil content and high carvacrol rate in the essential oil. It is intensely collected from the upland of Alanya and Gazipaş a towns of Antalya province in Turkey and used for essential oil production. Therefore, natural marjoram populations have been decreased year after year. Cultivation of marjoram seems to be the most convenient way for conservation of wild marjoram populations. However, there are various genotypes and chemotypes within the wild marjoram populations such as high carvacrol and high linalool types. The aim of this study is to select for high drog yields, high essential oil and carvacrol contents from wild *O. majorana* populations grown in the flora of Antalya. In the preliminary studies, high essential oil and carvacrol type populations were identified and their seeds were collected. These seeds were germinated in the greenhouse and then transplanted to the experimental plot. Plant height, number of branches per plant, herbal yield per plant, dry matter rates, essential oil rates and amount of essential oil per plant were measured at the flowering stage of each plant. Plant heights varied between 15 cm and 45 cm, the number of branches varied between 1 and 8, herbal yields per plant varied between 7 g and 127 g, dry matter rates varied between 28% and 60%, essential oil rates varied between 2.1% and 5.7%, amount of essential oil per plant varied between 0.15 ml and 0.85 ml. The results showed that great variations exist among the wild populations of *O. majorana.* Also, essential oil rate was negatively correlated with plant height and herbal yield. **Acknowledgements:** This research was supported by the Scientific and Technological Research Council of Turkey.

PN111 Determination of some traits in cultivated *Melissa officinalis* subsp. *altissima*

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There are three subspecies of *M. officinalis*: subsp. officinalis, subsp. inodora and subsp. altissima; however, only subsp. officinalis has commercial value and the characteristic lemony odor of lemon balm. Limited number of scientific reports on subsp. altissima were exist, although it's essential oil could have some potential as antifungal etc. The aim of the study was to determine some plant traits such as fresh herbage yield, fresh leaf rate, fresh leaf yield, plant height, number of stem per plant, leaf/stem rate of *Melissa officinalis* subsp. altissima. *M. officinalis* subsp. altissima seeds collected from the natural flora of Antalya and germinated in a greenhouse during winter season. After sixty days, seedlings were transplanted to the experimental field of the Faculty with 50 cm – 30 cm row and intra-row spacing, respectively. The experiment was carried out in randomized plots design with three replications. Drip irrigation system was used for irrigation and weeding control was done by the use of hand tools. No fertilizers were applied to the plots. Plants were harvested in flowering time with pruning shears. According to the results, average plant height was found 77 cm, average number of stem per plant was found 91, average fresh herbage yield and leaf yield were found 1996 kg/da and 1062 kg/da, respectively, essential oil rate was 0.12% and major component in the essential was caryophyllene oxide with the average rate of 43.50%. **References:** [1] Basta A, Tzakou O, Couladis M (2005), Flavour Fragrance Journal 20: 642 – 644

PN112 Cultivation Studies of *Stevia rebaudiana* Bertoni in Turkey

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Stevia rebaudiana Bertoni which belongs to the Asteraceae family is a herbaceous perennial plant and native to Paraguay. *Stevia* leaves produce steviol glycosides (stevioside and rebaudiosides), zero calorie natural sweetener and may substitute sucrose as well as other synthetic sweeteners, being 200 – 300 times sweeter than sucrose. Adaptation studies in Mediterranean climate condition of Antalya in Turkey was started four years ago and, initially seed germination problem was solved using cross-pollination of stevia flowers. The aim of this study was to determine some plant traits such as plant height, plant weight, number of stem per plant, leaf yield, leaf/stem ratio, days for flowering, stevioside and rebaudioside A rates of stevia in the conditions of Antalya. The experiment was carried out in randomized plots design with four replications for two years. Row spacing and intra-row spacing applied were 65 cm and 45 cm, respectively. In the experimental plots, drip irrigation was used and weeding was controlled by using garden tools. No fertilizers were applied to the plots. Plants were harvested at the beginning of the flowering time using pruning shears. According to the results, average plant height was found 98 cm, average number of stem per plant was found 9, average plant weight value was recorded as 720 g, average leaf/stem ratio was 1.03, average green leaf yield per hectare was found 8.6 tonnes, average days for flowering was 160 days, stevioside and rebaudioside A rates was found as 8.2% and 3.5%, respectively. In conclusion, stevia could be cultivated successfully in the conditions of Antalya as a perennial crop. **References:** [1] Yadav, A. K., Singh, S., Dhyani, D., Ahuja, P. S. (2011), Canadian Journal of Plant Science, 91: 1 – 27.

PN113 Biological activities of endemic *Prangos hulusii*

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Prangos species have been traditionally used to stop bleeding and as a wound and scar healer, emollient, antifungal, antihemorrhoidal, antioxidant in Turkey (1). *P. hulusii* S.G. Şenol, H. Yıldırım & Ö. Seçmen is one of the newly identified endemic species and no researches on it has been done yet (2). This study was aimed to investigate cytotoxic, antioxidant and antibacterial capacity of aerial parts (AP) and roots (RP) of *P. hulusii* which is widely used in folk medicine. The AP and RP were extracted successively with petroleum ether, dichlorometan and methanol in Soxhlet apparatus (1). For antioxidant effect, inhibition of lipid peroxidation in liposomes, induced by Fe^{3+}/ascorbate system, scavenging effect on $DPPH^{\cdot}$ and superoxide radicals and reducing power were analysed. The antibacterial activity was investigated against Gram(+) and Gram(-) reference bacterias (*S. aureus*, *E. coli*, *K. pneumonia*, *B. cereus*, *P. vulgaris*) and yeast *C. albicans* by using standard microbroth dilution and disk diffusion methods. Also, the cytotoxic activities of the crude extracts were examined by LDH (Lactate dehydrogenase) and MTT (mitochondrial succinate dehydrogenase) assays in the rat kidney epithelial cell line. As a result, AP extracts showed better antioxidant activity than RP extracts with the strongest potency for the methanol, followed by dichlorometan extracts. In the MTT test, the crude extracts were not cytotoxic at studied concentrations (0.5 – 0.125 mg crude extract/ml for AP, 0 – 0.2 mg crude extract/ml for RP), however, LDH test results showed that only petroleum ether extracts demonstrated low cytotoxicty. The antimicrobial activities of all extracts were observed against *S. aureus*, *B. cereus* and yeast. **References:** [1] Razavi M.S., Zarrini G., Zahri S., Mohammadi S., Biological activity of *Prangos uloptera* DC. Roots, A Medicinal Plant from Iran, Nat Prod Res, 24,9,797 – 3,(2010) [2] Şenol S.G., Yıldırım H., Seçmen Ö.S., *Prangos hulusii* sp.nov. from West Anatolia, Turkey. Nord J Bot,29,402 – 7(2011).

PN114 Kavalactones as inhibitors of advanced glycation endproducts (AGEs) formation

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The end-products of a complex series of non-enzymatic reactions involving glycation of proteins are the AGEs. Elevated levels of AGEs are associated with diabetic complications (nephropathy, retinopathy, cataract), atherosclerosis, neurological disorders and the normal ageing process. In this study DL-kawain (**1**), methysticin (**2**) and dihydromethysti-

cin (3), all belonging to the group of kavalactones, were identified as AGEs inhibitors. With IC_{50} values of 0.87 ± 0.02 mM and 0.90 ± 0.03 mM for 1 and 2, respectively, the compounds inhibited the *in vitro* protein glycation significantly better than aminoguanidine (IC_{50}= 4.62 ± 0.23 mM; p= 0.01). Compound 3 showed lower inhibitory activities (IC_{50}= 11.28 ± 0.87 mM). Furthermore, compounds 1 and 2 inhibited the formation of fructosamine, which is an intermediate in the process of AGEs formation. Moreover, 1 and aminoguanidine prevented AGEs formation by chelating Cu^{2+} and Fe^{3+}. However, these compounds showed less entrapment of the reactive carbonyl species (RCS), glyoxal and methylglyoxal, compared to aminoguanidine. These data indicate that kavalactones prevent early and advanced glycation, partly through metal chelation, and partly through the entrapment of RCS.

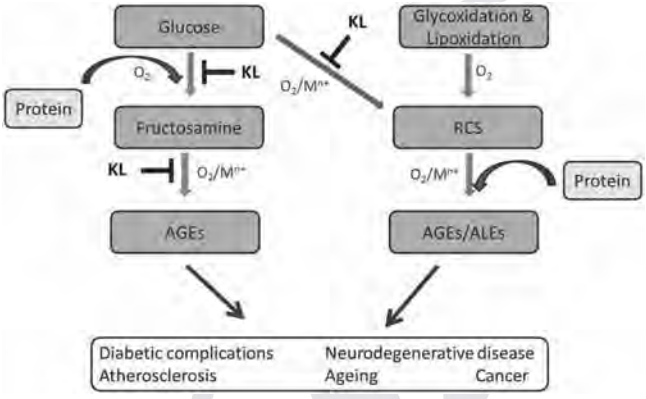

Fig. 1

PN115 Synergistic inhibition of Influenza replication cycle with *Echinacea purpurea* and *Sambucus nigra*

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Introduction Hemagglutinin and neuraminidase enzyme present therapeutic targets for the prevention and treatment of influenza. *Echinacea purpurea* extracts have been shown to inhibit hemagglutinin activity and viral infectivity [1]. In this study we examined the possibility that *Sambucus nigra* juice could additionally affect other parameters of influenza replication cycle and consequently enhance antiviral activity. **Methods** An ethanol extract (65% V/V) from freshly harvested *E purpurea* herb and roots (**EF**), the juice from *Sambucus nigra (SAM)* fructus and a fixed combination thereof (**Echinaforce® Hotdrink, *EF+SAM***), were all assayed for interaction with viral hemagglutinin and neuraminidase activities, and inhibition of influenza virus (H1N1) replication in MDCK cells as described in [1]. **Results** Lower concentrations of EF+SAM (0.0005% EF/0,02%SAM) were required to inhibit 50% of H1N1 replication than the single extracts with 0.006% for EF and 0.41% for SAM indicating super-additive effects of the EF+SAM combination. We observed varying kinetics, with early blockade of infection by Echinacea, while Sambucus' activity developed over time: EF activity was attributed to hemagglutinin blockade, indicating an early stage interference with infection. In contrast, SAM had no measurable activity on receptor interaction (HA activity) but inhibited activity of neuraminidase by 40% and more strongly than EF at the same concentration of 0.04%. **Conclusion** *E purpurea* ethanol extract and juice of *Sambucus nigra* have complementary points of activity to inhibit the influenza replication cycle. In combination the extracts inhibit both hemagglutinin and neuraminidase activities, i.e. virus entry and release of progeny virions, demonstrating synergistic effects in inhibition of influenza virus. **Reference:** [1] Pleschka S, Stein M, Schoop R, Hudson JB. Virology Journal 2009;6:197.

PN116 Mood enhancement by bergamot (*Citrus bergamia* (Risso) Wright & Arn.) volatile oil vapor with regards to personality and lifestyle related changes in salivary cortisol levels: A randomized cross-over trial

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In spite of still insufficient clinical evidence, bergamot essential oil (BEO) is widely used in aromatherapy for chronic stress and anxiety. In a randomized cross-over trial, data were collected under three testing conditions – rest (R); rest + water vapor (RW); rest + water vapor + BEO (RWB) (GC-MS: 45.45% limonene, 23.10% of linalyl acetate, 8.05% γ-terpinen, 7.25% β-pinene, 6.50% linalool, 1.35% α-pinene, 0.35% geranial) – for 15 min each in 42 healthy women, who had to fill out an initial personality traits and lifestyle questionnaire before the trial. Heart rate variability was recorded continuously, calculating its high-frequency component (HF), and saliva samples were collected for latter determination of salivary cortisol (CS) levels via ELISA after each test, while the subjects were given 10 min of rest and an additional 10 min to fill out psychological questionnaires. In comparison to the pure water placebo, the BEO vapor data show a significant increase in HF heart rate components (p = 0.009), indicating increased activity of the autonomous nervous system, as well as a decrease in confusion (p = 0.035) and fatigue (p = 0.042), paired with enhanced vigor of mood (p = 0.017). Change patterns of CS values were highly correlated with basic personality traits of the individual volunteers: CS levels decreased in 32 subjects (CS-D group) but increased in the remaining 10 (CS-I group). When comparing the lifestyles of this CS-I group with those of the of the CS-D group, the former were significantly more ordered (t =-2.08, p = 0.044) and values for extraversion (t = 1.92, p = 0.048), and optimism (t = 2.17, p = 0.035) were higher, while their feelings of fatigue (t =-2.61, p = 0.012) were lower. The data at hand indicate that BEO, when inhaled after dispersion in water vapor, exhibits swift psychological and physiological effects favorable for the therapy of chronic fatigue, stress, and anxiety, but also emphasize the influence of individual lifestyle on these conditions.

PN117 Leoligin formation in transformed hairy roots of Edelweiss (*Leontopodium alpinum* Cass.)

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Lignans constitute a group of plant metabolites of which several compounds are known to possess pronounced biological activities. Leoligin, a lariciresinol derivative, has been recently isolated from the underground parts of Edelweiss (*Leontopodium alpinum* Cass., Asteraceae) and was shown to inhibit in vitro leukotriene biosynthesis and intimal hyperplasia of venous bypass grafts (Reisinger et al. 2009). As yet a synthesis of this complex structure is not available, and due to the low content (between 0.005 and 0.010 w%) in the small roots of field grown Edelweiss acquisition of the compound is laborious. Thus, the biotechnological production of leoligin might be an interesting alternative. Edelweiss shoot cultures were transformed with *Agrobacterium rhizogenes* wild type strains to obtain hairy roots. A fast growing clone was treated with elicitors (silver nitrate, methyl jasmonate, yeast extract, and elevated sucrose concentration) and leoligin formation was quantified by a validated HPLC/PAD assay (w% of dry plant material). Untreated hairy roots were found to contain 0.0094 ± 0.0004 w% leoligin. By elicitation with either 200 μM methyl jasmonate or 6% sucrose lignan content could be nearly doubled to 0.0162 ± 0.0008 w% and 0.0161 ± 0.0018

w%, respectively, although elicitor treatment led to slightly reduced biomass increase. After further clone selection and optimization of growth parameters the procedure could be an attractive option for the continuous, field culture-independent production of leoligin. **Reference:** [1] Reisinger U, Schwaiger S, Zeller I, Messner B, Stigler R, Wiedemann D, Mayr T, Seger C, Schachner T, Dirsch VM, Vollmar AM, Bonatti JO, Stuppner H, Laufer G, Bernhard D. (2009) Leoligin, the major lignan from Edelweiss, inhibits intimal hyperplasia of venous bypass grafts. Cardiovasc Res 82(3): 542 – 9.

PN118 Anti-*Helicobacter pylori* activity of local edible plants

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Helicobacter pylori, gram negative bacteria, are mainly located in human stomach and, recognized as the cause of gastritis, dyspepsia, peptic ulceration and gastric carcinoma.[1] Eradication of *H. pylori* is hard to achieve and requires multiple antibiotic regimens. The growing problem of antibiotic resistance by the microorganism demands the search for novel compounds from plants. This study aimed at evaluating the anti-*Helicobacter pylori* activity of edible plants used in local area of Thailand. The *in vitro* antibacterial activity of a total of 57 methanolic extracts from dietary plants and spices were investigated by disc diffusion method against *H. pylori*. The inhibition strength of extract specimens can be classified in 4 groups which are strong, moderate, mild and no activity. The specimens that showed strong activity were then evaluated the MIC against *H. pylori* using agar dilution technique. It was found that the methanol extract of *Zingiber officinalis*, *Curcuma parviflora*, *Peperomia pellucida*, *Polygonum odoratum*, *Gymnema inodorum*, *Plectranthus amboinicus*, *Spondias pinnata* and *Marsilea crenata* revealed activity against *H. pylori* with MIC 2.5 μg/ml whereas MIC of amoxicillin, positive control, is 7.81 μg/ml. The results showed the possibility of considering local edible plants a chemopreventive agent for peptic ulcer or gastric cancer. **Reference:** [1] Kandulski A, Selgrad M and Malfertheiner P. (2008). DIGEST LIVER DIS. 40: 619 – 626.

PN119 Phytochemical assessment of the effect of stimulated *in vitro* multiplication on the metabolic profile of *in vitro* cultured *Hypericum richeri* and *Artemisia alba*

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In vitro secondary metabolite production requires a fine balance between biomass formation and expression of the biosynthetic capacity of the species. Comparison of the metabolite spectra of the *in vitro* to *ex situ* material is a crucial benchmark in this process. HPTLC methods known from similar plant species from the *Artemisia* and *Hypericum* genera [1, 2] have been adapted to extracts with varying polarity of different plant parts from *ex situ* and *in vitro* samples of *Artemisia alba* and *Hypericum richeri*. The main differences and similarities of the secondary metabolite and bioactive constituent profiles between *in situ* and *in vitro* produced plant material were assessed. The results of this study revealed that *A. alba* can be maintained successfully long-term in medium lacking plant growth regulators (PGR) whereas *H. richeri* requires cytokinin supplementation in order to stimulate axillary bud multiplication and sustain growth *in vitro*. It was also established that N^6-Benzyladenine (BA) strongly stimulated multiplication index and its individual application led to inhibition of rooting for both species. While the combination of BA and indole-3-butiric acid was found to be favorable for both biomass and polyphenolics stimulation in *A. alba*, enhanced growth led to the drop of polyphenols and flavonoids in *H. richeri*. At the same time hypericins were observed in significant levels *in vitro*. Further research is in progress to clarify the distinctive features of the biochemical and physiological response to PGR treatment as a model system for affecting antioxidant metabolites production *in vitro*. *Acknowledgments:* This work was supported by the Swiss National Science Foundation in the framework of the Bulgarian-Swiss Research Programme (BSRP, grant No. IZEBZ0_142989; DO2 – 1153) **References:** [1] Proksch P and Wissinger-Gräfenhahn U. Artemisia. In: Blaschek W et al. (Eds.) Hager ROM 2011. Springer Heidelberg [2] European Pharmacopoeia 7.7 online edition

PN120 Precursor feeding and elicitation improve mitragynine production in *Mitragyna speciosa* shoot culture

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Mitragyna speciosa (Roxb.) Korth. [Rubiaceae] is a unique source of mitragynine, an opioid agonist and possesses an analgesic effect by non-selective binding to opioid receptors. The present study shoot culture from axillary buds were established and cultured in the liquid McCown woody plant medium (WPM) supplemented with 2 mg/L thidiazuron, 1 mg/L benzyladenine and 20 g/L sucrose. To improve mitragynine production in the shoot culture, feeding of precursors and elicitors was investigated. Tryptamine, loganin and tryptamine/loganin (final concentration of 0.2, 0.4 mM) were fed on the day of subculture and harvested at day 14 and 21 of culture. The results showed that feeding of tryptamine affected the mitragynine content rather than loganin. However, a combination of tryptamine and loganin (at 0.4 mM of each) enhanced mitragynine production to 0.364 ± 0.009 mg/g DW (after 14 days; $P< 0.01$) and 0.273 ± 0.015 mg/g DW (after 21 days; $P< 0.05$) when compared to control (0.169 ± 0.019 mg/g DW). Plant hormone-like elicitors including jasmonic acid (0 – 100 μM), abscisic acid (0 – 100 μM) and salicylic acid (0 – 500 μM) were selected. The optimal concentration and optimal time exposure of each elicitor were designed using the response surface methodology (RSM) and central composite design (CCD). The equations, obtained from RSM and CCD were used for calculation of optimal conditions in each elicitor. At optimal conditions, the elicited shoot culture with jasmonic acid (100 μM; 48 h) increased mitragynine production (0.482 ± 0.021 mg/g DW) about 2.4 times higher than the control (0.188 ± 0.030 mg/g DW). In addition, abscisic acid (50 μM; 24 h) and salicylic acid (500 μM; 48 h) increased the mitragynine production to 0.273 ± 0.007 mg/g DW and 0.279 ± 0.016 mg/g DW, respectively when compared to the control (0.116 ± 0.008 mg/g DW).

PN121 Synthesis and biological evaluation of tetrahydroisoquinoline skeletion from *Ancistrocladus tectorius*

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Ancistrocladus tectorius is this plant used in traditional medicine to treat dysentery, malaria and has additionally been found to exhibit antiviral, anti-HIV, antitumoral activities. In this plant found in Ancistrotectorin (**1**) which exhibits anti-spasmolytic for smooth muscle similar to those of verapamil and papaverine. It structure similar to tetrahydroisoquinoline and was used as a template in this synthesis products. In this study to synthesis of structure of 1,2,3,4-tetrahydroisoquinoline to mimic structure of tetrahydroisoquinoline found in *Ancistrocladus tectorius*. To synthesize the tetrahydroisoquinoline have variation position 1 substituted with 4-Cl-phenyl (**2**) and 4-NO_2-phenyl (**3**) group on tetrahydroisoquinoline skeleton. These target compound were prepared via the Pictet-Spengler reaction to give two diastereoisomers of target compounds **2** and **3**. The major (**2a** and **3a**) and minor (**2b** and **3b**) products were isolated and structure elucidated with 1D and 2D NMR spectroscopy to be *cis*- and *trans*-isomers, respectively. The synthesized compounds as **2a** and **2b** were primary screened for anticonvulsant activity via Pentylenetetrazol (PTZ)-induced seizure model in mice. The activity result showed that administration of *cis*-isomer (**2a**) is significantly increased time latencies for the onset of myoclonic jerk ($p < 0.05$). Moreover, *cis*-isomer seems to be a good arrangement for the anticonvulsant activity. And these finding indicate that the simple structure of 1,2,3,4-tetrahydroisoquinoline might be a good candidate as a new therapy for epilepsy and more derivatives. And next, more derivatives were synthesizes such as **3a** and **3b** and anticonvulsant were also study.

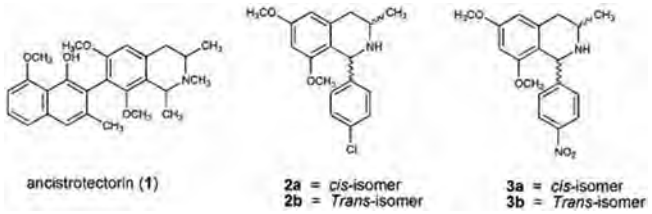

Fig. 1

PN122 Germination and growth characteristics of *Dendropanax morbifera* Lev. by sowing methods and shading conditions

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Korea Forest Research Institute, Southern Forest Resources Research Center, JinJu (660 – 300), South Korea

Dendropanax morbifera LEV. belongs to family Araliaceae is a subtropical broad-leaved evergreen tree distributed in Korea, Japan, China and Taiwan. *D. morbifera* is highly valuable forest resource used not only as ornamental trees but also golden lacquer and medicinal materials in Korea. However, natural habitats of *D. morbifera* are locally distributed in warm-temperature region of Korea. This study was carried out to obtain basic information for the improvement conditions of germination rate according to the various sowing methods and proper shading conditions to produce superb seedlings. We measured the seed germination rate (GR) and germination performance index (GPI) of *D. morbifera* under the various conditions of sowing time, pre-chilling and PEG (polyethyleneglycol) treatments to find out the promotive way of germination. In addition, we had observed the morphological-physiological changes of *D. morbifera* seedlings by different shading conditions. As a result, the seed of *D. morbifera* was showed the highest germination rate when the seeds were sowed in March with more than 30 days pre-chilling treatment and -2.0Mpa PEG treatment. However there was no statistical difference between PEG treatments. *D. morbifera* showed the highest values in the leaf area (TLA), H/D ratio, chlorophyll contents and photosynthetic capacity at 95% shading condition and those factors were increased with a statistical significance by decreasing light level. On the other hand, at 50% shading condition, *D. morbifera* had maximum value in dry-weight of root and seedling quality index (SQI) but minimum T/R ratio. Those results suggested that the optimum shading condition of *D. morbifera* for the vigorous seedling was approximately 50% level.

PN123 Anti-inflammatory depsides from *Cetrelia monachorum* potently targeting mPGES-1, 5-LO and NF-κB

Oettl SK[1], Gerstmeier J[2], Wiechmann K[2], Bauer J[3], Atanasov AG[4], Heiss EH[4], Waltenberger B[1], Boustie J[5], Dirsch V[4], Stuppner H[1], Werz O[2], Rollinger JM[1]
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Lichens, often underestimated and still underexplored with respect to pharmaceutical lead discovery, provide a vast diversity of small chemical entities with a variety of reported bioactivities. Based on a previously performed pharmacophore-based virtual screening, we identified lichen constituents as potent microsomal prostaglandine E_2 synthase 1 (mPGES-1) inhibitors [1]. Here, we focused on further *in vitro* anti-inflammatory activities of lichen compounds. An *in vitro* screening of 17 Alpine lichen species for inhibition of 5-LO, mPGES-1 and NF-κB revealed *Cetrelia monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb. as promising source for novel anti-inflammatory leads. Phytochemical investigation of the ethanolic crude extract resulted in the isolation and identification of 11 constituents, belonging to depsides and derivatives of orsellinic acid, olivetolic acid and olivetol. The two depsides imbricaric acid and perlatolic acid exerted inhibitory activities on mPGES-1 (IC_{50} 1.9 and 0.4 μM, resp) and 5-LO as demonstrated in a cell-based assay (IC_{50} 5.3 and 1.8 μM, resp) and on the purified enzyme (IC_{50} 3.5 and 0.4 μM, resp). Dual inhibition of mPGES-1 and 5-LO provides safer and more effective anti-inflammatory properties [2]. Furthermore, the two main constituents, imbricaric acid and perlatolic acid, quantified in the extract with a content of 15.22% and 9.10%, resp, showed significant inhibition of TNF-α-induced NF-κB activation in luciferase reporter cells with IC_{50} values of 2.0 and 7.0 μM, resp. These findings attest imbricaric acid and perlatolic acid a pronounced threefold anti-inflammatory profile [3], which warrants further investigation on their pharmacokinetics and *in vivo* efficacy. **Acknowledgements:** Supported by the Austrian Science Fund (S10703, S10704). **References:** [1] Bauer J et al. ChemMedChem 2012 7: 2077 – 81 [2] Radmark O, Samuelsson B J Intern Med 2010 268: 5 – 14 [3] Oettl SK et al. 2013 submitted.

PN124 Synergy and antagonism of active constituents in a complex herbal formulation on metabolic regulation at a transcriptional level

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Gene expression profiling was conducted on the human neuroglial cell line, T98G, after treatment with either a complex herbal formulation (ADAPT-232) or its constituents, which included extracts of Eleutherococcus senticosus root, Schisandra chinensis berry, and Rhodiola rosea root or several individual constituents, including eleutheroside E, schizandrin B, salidroside, triandrin, and tyrosol. The concentration at which the compounds were tested strongly influenced both the intensity of the cellular response and the profile of differentially expressed genes. Combining two or more active substances in one mixture significantly changed deregulated gene profiles: synergetic interactions resulted in activation of genes that none of the individual substances affected; antagonistic interactions resulted in suppression of some genes that had been activated by the individual substances. These interactions may influence transcriptional control of metabolic regulation, on both the cellular and the whole organism levels. This study was the first to demonstrate that combining active substances with different deregulated gene array profiles and intracellular networks could produce a new substance with unique pharmacological characteristics. Thus, the mixture of two chemical substances could produce a qualitatively new substance, biologically different from its constituents. Presumably, this phenomenon could be used to eliminate undesirable effects (e.g. toxic effects) and increase the selectivity of pharmacological interventions.

Erratum

PM2 Anti-*Helicobacter Pylori* Activities of Nigerian Propolis (Bee Product)

Alaribe Chinwe S[1], Rastrelli L[2], Sunday A[3], Emmanuel O[4], Sola I[5], Herbert C[1]
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Erratum for Planta Medica 10/2013:
The name of the second author of the congress-abstract "Anti-*Helicobacter Pylori* Activities of Nigerian Propolis (Bee Product)" (Planta Medica 2013; 79 (10):809–896) was changed to L. Rastrelli.

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