BOTANICAL INGREDIENT ADULTERATION

HOW SOME SUPPLIERS ATTEMPT TO FOOL COMMONLY USED ANALYTICAL TECHNIQUES

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American Botanical Council

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Innsbruck, Austria
Is Adulteration a Problem in Europe?

EU supplement law ‘among world's strictest,’ as study finds two thirds adulterated

By Will Chu  

An industry group declares the EU legal framework for food supplements ‘among the strictest in the world,’ in response to a study that finds almost two-thirds of supplements contain pharmacological active substances or plant toxins.
Taking Advantage of Unclear Taxonomy – *Euphrasia officinalis* Identification Challenges

- Species hybridize frequently

- Self-pollination and insect pollination → species may exhibit highly variable morphology (e.g., *E. minima*)

- Morphological distinction criteria often blurred (e.g., *E. rostkoviana* vs. *E. montana* or *E. versicolor*)

- Botanists use different scientific names for the same species

*Euphrasia rostkoviana* (syn. *E. officinalis* subsp. *rostkoviana*)
The Peculiar Case of *Euphrasia odontites*

- Included in the Linnaean herbarium as *Euphrasia odontites*
- Species named *Euphrasia serotina* by Jean-Baptiste Lamarck in 1778 (illegitimate)
- Reclassified as *Odontites vulgaris* by Conrad Moench in 1794
- Reclassified as *Odontites serotinus* by Barthélemy Charles Joseph Dumortier (1827)
- Reclassified as *Odontites vernus* subsp. *serotinus* in 1893 by François Marie Louis Corbière

Common name: red bartsia
Still sold as *Euphrasia odontites* or eyebright today
HPTLC Investigation of Commercial Eyebright Samples

• 28 botanical samples collected in the wild, including 25 *Euphrasia* spp. samples, *Odontites lutea*, *O. viscosus*, and *Bartsia alpina*

• 32 commercial samples analyzed: 25 bulk materials and 7 finished products (USA: 28; Europe: 4)

• Bulk materials originating in Bulgaria, Croatia, Macedonia, Poland, and Ukraine
Results

Mobile phase: Dichloromethane, methanol, water (60:40:4)

Samples:
B1-B25: Bulk samples
P1-P7: Dietary supplements
A: Aucubin
Er: Euphrasia rostkoviana
Es: Euphrasia stricta
Ol: Odontites lutea
Ov: Odontites viscosus

Mobile phase: Ethyl acetate, acetic acid, formic acid, water (100:11:11:26)

Bulk materials: 8 Odontites spp. (all from USA), 13 Euphrasia spp., 4 weak or blank
Dietary supplements: 4 Euphrasia, 3 weak or blank
Absorption at specified wavelength is not specific to an individual marker compound

Applications:
- Total anthocyanins in bilberry, blueberry, elderberry, etc.
- Hypericin content in St. John’s wort
- Total proanthocyanidin content in cranberry, grape seed, etc.
Cranberry: Supply Chain

Cutting of fruit

Pumping berries and water

Filling truck

Removing stems

Sugar-infused cranberry ready for drying

Juice press
Adulteration of Cranberry (*Vaccinium macrocarpon*) Extract

- In 2017, cranberry is the third best-selling herb in the US mass market and # 13 in the US natural channel with sales over US$ 75 Mio*

- Beneficial effects for preventing urinary tract infection reportedly due to proanthocyanidins (PACs), in particular A-type

- Cranberries at 100 g fresh weight provide ca. 420 mg total flavan-3-ols, of which 56% (235 mg) are polymers**

- Other plants contain higher amounts of PACs and present cheaper sources

*Smith T, email communication June 19, 2018.

Adulteration of Cranberry Extract

*Pinus massoniana*  
B-type

*Vitis vinifera*  
B-type

*Arachis hypogaea*  
A-type and B-type

*Oryza sativa*  
A-type and B-type

Proanthocyanidins: Complex mixtures – polymers tend to elute as “mole hills” with HPLC

Procyanidin A2  
Procyanidin B1
Specificity: Cranberry Extract

UV/Vis spectrophotometry (DMAC)

MALDI-TOF

MALDI-TOF spectrum of cranberry PACs (Feliciano et al., Food Chem. 2012; 135: 1485-1493)
Adulteration of Cranberry (*Vaccinium macrocarpon*)

**cranberry juice extract**

**Product Details**
- **English name:** bilberry extract, cranberry extract
- **Latin Name:** Vaccinium vitis-idaea Linn.
- **CAS No.:** 84082-34-8
- **Molecular formula:** C27H31O16
- **Molecular Weight:** 611.53
- **Active ingredients:** Anthocyanidin
- **Specification:** 10%, 15%, 20%, 25%
- **Use Part:** fruit
- **Appearance:** Dark purple fine powder
- **Mesh size:** 80 Mesh
- **Test Method:** UV

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**Quick Details**
- **Type:** Herb
- **Part:** Fruit
- **Place of Origin:** Shaanxi
- **Model Number:** 25%
Specificity: Bilberry Extract

UV/Vis spectrum of amaranth dye

HPLC-UV chromatogram (535 nm) of bilberry extract according to the European Pharmacopoeia. Image provided by Indena S.p.A. (Milan, Italy).
Bilberry Extract Adulterants

Anthocyanidin-rich extracts from

- Bog bilberry (*Vaccinium uliginosum*)
- Lingonberry (*V. vitis-idaea*)
- Blueberry species (*V. angustifolium, V. corymbosum*)
- Cranberry (*V. oxycoccos and V. macrocarpon*)
- Raspberries (*Rubus* spp., Rosaceae)
- Wild cherry (*Prunus avium*, Rosaceae)
- Black chokeberry (*Aronia melanocarpa*, Rosaceae)
- Mulberry species (*Morus australis, M. nigra*, Moraceae)
- European elder (*Sambucus nigra*, Adoxaceae)
- Black soybean (*Glycine max*, Fabaceae)
- Black rice (*Oryza sativa*, Poaceae)
- Amaranth dye
- Charcoal
Same issue with...

Sambucus nigra subsp. canadensis
(American Elderberry)

Aronia melanocarpa
(Image provided by Gayle Engels)

Euterpe oleracea
(www.tradewindsfruit.com)
GC-FID

Applications: Analysis of volatile compounds (compounds of higher polarity after derivatization)

*Potential issues with GC-FID method:*

- Use of marker compound(s) that are not specific for plant/plant part
- Adulterant may not be soluble in solvent used for sample preparation
- Adulterant may not be volatile

*Common ways to fool GC-FID:*

- Addition of same/similar compounds from other plant sources
- Admixture of nature-identical marker compounds
Tea Tree Oil
Melaleuca alternifolia

Reported adulterants:

- Waste stream products after rectification of camphor (Cinnamomum camphora), eucalyptus (Eucalyptus spp.), or pine (Pinus spp.) oils
- Monoterpenes obtained via fermentation or semi-synthesis (e.g. catalytic conversion of sabinene to terpinen-4-ol)

Adulteration with terpinen-4-ol may be detected by the presence of $p$-menth-1-ene, $t$-pinocarveol and $p$-menth-3-ene
Tea Tree Oil Laboratory Guidance Document

By Stefan Gafner, PhD and Ashley Dowell

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Keywords: Adulteration, Eucalyptus globulus, eucalyptus oil, Melaleuca alternifolia, Melaleuca linearifolia, tea tree oil, white camphor oil

1. Purpose

Tea tree oil (TTO) is the essential oil of tea tree (Melaleuca alternifolia or M. linearifolia, Myrtaceae). Adulteration of TTO has become more apparent in recent years. Adulteration occurs with single essential oil components (e.g., sabinene from pine oil), waste products derived from other essential oils such as pine (Pinus spp., Pinaceae), eucalyptus (Eucalyptus spp.), and other chemotypes of tea tree oil (e.g., white camphor oil).
Tea Tree Oil Laboratory Guidance Document

Detailed chemical composition of TTO and its adulterants

Assessment of:

- Macroscopic, microscopic, and organoleptic tests
- Genetic assays
- Physicochemical tests
- Chemical Analysis
  = HPTLC
  = Infrared (IR, MIR, NIR)
  = Gas Chromatography

Input from 13 peer-reviewers

HPLC-UV/Vis

Applications: Almost any botanical ingredient

Potential issues HPLC-UV/Vis method:

- Use of marker compound(s) that are not be specific for plant/plant part
- Adulterant may not be soluble in solvent used for sample preparation
- Adulterant lacks chromophore
- Highly polar adulterant

Common ways to fool HPLC-UV/Vis:

- Addition of same/similar compounds from other plant sources
- Addition of extracts from other plant parts
- Admixture of nature-identical marker compounds
Adulteration of Ginkgo biloba Leaf Extract

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Keywords: adulterant, adulteration, Ginkgo biloba, ginkgo leaf extract, Japanese pagoda tree, Japanese sophora, kaempferol, quercetin, rutin, Sophora japonica, Styrphnolobium japonicum

Goal: The goal of this bulletin is to provide timely information and/or updates on issues of adulteration of ginkgo (Ginkgo biloba) leaf and ginkgo leaf extracts to the international herbal industry and extended natural products community in general. It is intended to give a brief overview on the occurrence of adulteration, known adulterants and analytical means to detect them, the market situation, and perspectives for the future.
Pharmacopeial (USP) Method to Determine Total Flavonol Glycosides in Ginkgo Extracts

- Quercetin-, keampferol-, and isorhamnetin-glycosides are subjected to hydrolysis
- Resulting quercetin, keampferol, and isorhamnetin are quantified by HPLC-UV
- Total flavonol glycosides are quantified using conversion factor (2.504 for quercetin, 2.588 for kaempferol, and 2.437 for isorhamnetin)
- Adulteration by addition of pure quercetin, kaempferol, or rutin, or rutin-rich extracts from other sources (buckwheat, Japanese sophora)
## Adulteration of Ginkgo biloba extracts

Summary of 21 investigations into the authenticity of commercial extracts from 2003-2019

<table>
<thead>
<tr>
<th></th>
<th>Sample number</th>
<th>Adulterated samples</th>
<th>Adulteration [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples*</td>
<td>418</td>
<td>192</td>
<td>45.9</td>
</tr>
<tr>
<td>Asia</td>
<td>35</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>Australia/New Zealand</td>
<td>14</td>
<td>6</td>
<td>42.9</td>
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<tr>
<td>Europe</td>
<td>174</td>
<td>88</td>
<td>50.6</td>
</tr>
<tr>
<td>Europe (herbal medicine)*</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Europe (food supplement)*</td>
<td>85</td>
<td>49</td>
<td>55.3</td>
</tr>
<tr>
<td>North America</td>
<td>69</td>
<td>37</td>
<td>53.6</td>
</tr>
</tbody>
</table>

*Some authors did not detail the origin of the analyzed products, or distinguish among food supplements and herbal medicine
Same issue with...

Crataegus spp.

Passiflora incarnata
Adulteration of Turmeric Root/Rhizome and Extracts

- Turmeric extracts contain diarylheptanoids with a plethora of reported health benefits
- Best-selling herbal dietary supplement in Natural channel, 5th in mass market in U.S. with sales above US $ 80 million*
- Main use is to treat inflammatory conditions

Adulteration of Turmeric Root/Rhizome and Extracts

• Dried turmeric has a rough surface and dull color: roots are often polished to look more appealing
• After polishing, visual aspect of roots may be improved by wet or dry coloring (turmeric powder, undeclared synthetic colorants, e.g. lead chromate, metanil yellow)
• Other *Curcuma* species may be used as substituents
• Synthetic curcumin added to comply with standardization requirements

*Curcuma longa* *Curcuma zedoaria* *Curcuma-zanthorrhiza*
Detection: Carbon Isotope Measurements

- Steady-state between incorporation of $^{14}$C (via photosynthesis using CO$_2$) into plant and its decay (half-life of 5730 years)
- After plant dies, no further incorporation of $^{14}$C

  - Concentration of $^{14}$C is highest in living plants
  - Fossil fuel-derived products have little or no $^{14}$C

- Certain plants (grasses, corn, sugar cane) incorporate $^{13}$C at lesser amounts than others

  - $^{13}$C/$^{12}$C ratio can be used to determine corn– or sugar cane-derived materials
Is Synthetic Curcumin/Piperine Involved in Italian Hepatitis Outbreak?

Curcuma ed epatite: salgono a 21 i casi individuati. Segnalati 19 diversi integratori alimentari, ma ancora nessuna spiegazione

Continuano le segnalazioni di casi di epatite colestatica acuta, un'intossicazione del fegato che non è infettiva né contagiosa, e con esse quelle degli integratori alimentari a base di curcuma e curcumina. Al 20 giugno, erano 21 le persone colpite individuate dall'Istituto superiore di sanità.
UHPLC Analysis of 50% MeOH Extracts of Batches A+B
Reference Standards: Curcumin, Piperine, and Vitamins (B1, B2, and B6)

Detection wavelength: 254 nm

- **Piperine**
- **Curcumin**
- **Vit B6**
- **Vit B2**
- **Vit B1**

**Curcumin_SmpB (50% MeOH)**: Piperine/Curcumin ~7:1 (at 254 nm)

**Curcumin_SmpA (50% MeOH)**: Piperine/Curcumin ~1:1.5 (at 254 nm)

Net relative ratio piperine/curcumin ~11-fold ratio difference (at 254 nm)

Image provided by Guido Pauli, University of Illinois at Chicago
(q)HNMR Analysis of Samples A and B, aka “Yellow Tablets”

- “Yellow tablets” do not contain natural desmethoxy-curcuminoids
- “Yellow tablets” contain undeclared aromatic component

![HNMR Spectra](image)

Natural Bisdesmethoxy-curcumin

Natural Desmethoxy-curcumin

Natural Curcumin (“Purcumin”)

Investigated Tablets Sample B

Investigated Tablets Sample A
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