Challenges in Characterizing and Identifying Components in Botanical Products

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Department of Health and Human Services

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Disclaimer

- Views expressed are my own and do not reflect the views of ODS, NIH, or HHS
Known Bioactive Compounds in Foods

- Inherent to Foods
  - Alkaloids
  - Cyanogens
  - Glucosinolates
  - Lectins
  - Carbohydrase Inhibitors
  - Vicine/Convicine (Favism)
  - Phenolics
  - Non-protein Amino Acids
  - Peptides

- Accidental Ingestion
  - Mushroom Toxins

- Contaminants in Foods
  - Mycotoxins
  - Alkaloids
  - Shellfish toxins
Alkaloids

Caffeine-
LD50-127 mg/kg* mice, 50-500 mg/kg*, humans
Cassava (manioc)- *Manihot esculenta*

linamarin
Biogenic Amines

- Tyramine
- N-methyltyramine
- Octopamine
- Synephrine
## Biogenic Amines

<table>
<thead>
<tr>
<th></th>
<th>Syneprine (Mg/kg fresh)</th>
<th>Tyramine (Mg/kg fresh)</th>
<th>Me-Tyramine (Mg/kg fresh)</th>
<th>Octapamine (Mg/kg fresh)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. reticulata</td>
<td>125</td>
<td>1</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>(tangerine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. reticulata</td>
<td>280</td>
<td>1</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>(Mandarin or.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. aurantium</td>
<td>19</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>(sour orange)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musa paradisiaca</td>
<td>-</td>
<td>29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(banana)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
6'-7'-Dihydroxybergamottin
Grapefruit juice inhibits intestinal cytochrome P450 (CYP) 3A4
Glucosinolates

\[
\text{S-C}_6\text{H}_{11}\text{O}_5
\]

\[
\text{R-C} \equiv \text{N-OSO}_3^-
\]
Glucosinolates

3-Methylsulfinylpropyl-glucosinolate: Glucoiberin
Allyl-glucosinolate: Sinigrin
4-Methylsulfinylbutyl-glucosinolate: Glucoraphanin
3-Indolylmethyl-glucosinolate: Glucobrassicin
Figure 2. Representative chromatograms of A) fresh raw, B) boiled 5 min, and C) stir fried broccoli. GI = glucobrassicin; Pro = progoitrin; epi-Pro = epi-progoitrin; Sin = sinigrin; GB = glucobrassicin; GN = gluconasturtiin.
**Glucosinolates**

Hepatic, renal, Pancreatic lesions caused by OZT’s and nitriles

![Chemical structures]

Goitrin

Progoitrin

Thiocyanates and L-5-vinyl-2-thiooxazolidone = Goitrogens

• Activity of goitrin not reversible by dietary iodine
Carotatoxin- 10-20 mg/kg: LD50-100 mg/kg (i.p.), mice

$\text{H}_3\text{C} - (\text{CH}_2)_5\text{CH} - \text{CHCH}_2\text{C} - \text{C} - \text{C} - \text{CH}_2\text{CHCH} - \text{CH}_2$

OH
Fabaceae

Glycine max
Soy (Glycine max)

Soyasaponin II

Daidzin=daidzein-7-glucoside

Stachyose

Genistin=genistein-7-glucoside

Phytic Acid

Bowman-Birk Protease Inhibitor
"What we observe is not nature itself, but nature exposed to our method of questioning."

- Werner Heisenberg
Biological relevance

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rel. Efficiency</th>
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<tbody>
<tr>
<td>THBQ</td>
<td>1.00</td>
</tr>
<tr>
<td>BHA</td>
<td>1.67</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.18</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.01</td>
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</table>
## Cytotoxicity in Caco 2 cells

*(Inhibition of MTT reduction)*

<table>
<thead>
<tr>
<th></th>
<th>AJE</th>
<th>Quercetin</th>
<th>Rutin</th>
<th>Phloridzin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>50 µM</td>
<td>5 µM</td>
<td>5 µM</td>
<td>5 µM</td>
</tr>
</tbody>
</table>

- Addition of ascorbate or α-tocopherol abolished cytotoxicity.
- Postulated that formation of reactive polyphenol oxidation products at high levels responsible for cytotoxicity.
- Also postulated that AJE was less cytotoxic because the compounds protected each other.

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**Phloridzin**

*Pohl et al. 2004 *The Toxicologist* **78**,S-1: 213*
Hydroxylated flavonoids (quercetin, naringenin) usually inhibit P450 isozymes in vitro, nonhydroxylated flavonoids (flavone, nobiletin, tangeretin) stimulate P450 system in vivo and in vitro.
Quercetin

- Quercetin reduces expression of the amiloride-sensitive epithelial Na\(^+\) channel (ENaC) in salt-sensitive hypertensive rats (Aoi et al. 2004 *Biochem Biophys Res Commun* **315**:892)
  - ENaC is rate limiting step in Na\(^+\) reabsorption in distal segment of renal tubule
    - High-salt diet rats have elevated αENaC expression and BP
    - Quercetin blocked elevated expression of αENaC mRNA and BP elevation
    - Quercetin effect on αENaC not found in colon
      - Aglycone (colon) vs. glucuronide, sulfate after absorption?
Quercetin

- Quercetin (10 µM) inhibits insulin-stimulated methylglucose uptake by direct interaction with the facilitative glucose transporter GLUT4 in rat adipocytes (Strobel et al. 2005 *Biochem. J.* 386:471)
  - Does not inhibit protein tyrosine phosphorylation
- Quercetin non-competitively antagonizes the γ-aminobutyric acid C receptor subunit GABA\(_1\) (Goutman & Calvo, 2004 *Brit J Pharmacol* 141:717)
  - Quercetin active at ionotopic GABA receptors and other ligand-gated ion channels
St. John’s Wort

- 1814-1st American mention of toxicity of *H. perforatum* (to livestock)
- 1° photosensitivity
  - hypericin + light = lipid hydroperoxide *in vitro*
- SJW induced neuropathy, erythroderma
  - Hypericin-induced phototoxicity *in vitro*
  - long term effects?
- SJW/Indinavir
  - Herb/drug interactions
**SJW Constituents**

- Seven accessions of SJW collected in Tuscany (Bergonzi et al. 2001; *Drug Dev Ind Pharm* **27**:491)
  - Total flavonols: 4.6-15.9%
  - Total hypericins: 0.05-0.11%
  - Total hyperforins: 1.4-20.8%
Hypericum perforatum *L.*

- DAC ’86 (German Drug Codex)- not < 0.4% dianthrones of hypericin group, as hypericin
- DAC ’91: method change to correct for chlorophyll interference: results are 20% lower
- Hypericin MAO inhibitor *in vitro*
- 1996 trial showed hypericin-free but not hyperforin free products effective
- USP ‘99-SJW extract, not < 0.2% hypericin and pseudohypericin combined , not < 3.0% hyperforin (LC)
Are we having fun yet?

- Bioassay directed *H. perforatum* prep HPLC
- Flavonoid rich fractions - Antidepressant (FST)
  - Hyperoside, isoquercitrin, miquelianin, quercitrin, some hyperoside, astilbin
  - Isolated pure compounds tested
- All pure cmpds except quercetin, quercitrin, and astilbin active in FST ([Butterweck et al.](2000) *Planta Med.* **66**:3-6)
- 2002: new proprietary SJW extract product
SJW flavonoids

isoquercitrin
(quercetin 3-O-β-D-glucopyranoside)

miquelianin
(quercetin 3-O-β-D-glucuronopyranoside)

hyperoside
(quercetin 3-O-β-D-galactopyranoside)
Fig. 1 Uptake of different flavonoid-glycosides into Caco-2 cells with and without 50 μM MRP-2 inhibitor MK-571 (mean ± SE; n = 3 – 4).

Drug Discovery

- Natural Products Cancer Drug Discovery
- Bioassay Directed Fractionation to pure chemical entities
“Whole plant”

- *Berberis* spp.-marker=berberine
- Flavonolignan: 5'-methoxyhydnocarpin (5'-MHC) inhibitor of the MDR pump in *S. aureus*
- Prevents bacterial cells from eliminating berberine
"Of course you can't replicate my experiments. That's the beauty of them."
Analytical Methods and Reference Materials Program

- Quercetin dihydrate (7)
  Molecular Formula: $C_{15}H_{10}O_7 \cdot 2H_2O$, Formula Weight: 338.27, CAS Number: 6151-25-3
  - Q0125 $\geq 98\%$ (HPLC), powder (Sigma)
  - Q-112 solid (Sigma)
  - 83370 BioChemika, $\geq 98.0\%$ (HPLC) (Fluka)
  - 33,795-1 $\geq 95\%$ (Aldrich)
  - 17,196-4 98% (Aldrich)
  - 32782 >99% (Riedel-de Haën)
  - 69249 $\geq 99.0\%$ (HPLC) (Fluka)
# Quercetin Dihydrate

## (1200 mg)

<table>
<thead>
<tr>
<th>Company</th>
<th>HPLC Purity (%)</th>
<th>Water Content (%)</th>
<th>Residual Content (%)</th>
<th>Calculated Value (%)</th>
<th>Price</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>99.57</td>
<td>20.2</td>
<td>0</td>
<td>79.46</td>
<td>$550</td>
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<tr>
<td>B</td>
<td>99.46</td>
<td>0.41</td>
<td>0.4</td>
<td>99.12</td>
<td>$6500</td>
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<tr>
<td>C</td>
<td>ca 90</td>
<td></td>
<td></td>
<td></td>
<td>$1250</td>
</tr>
<tr>
<td>D</td>
<td>Not less than 98</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td>$1320</td>
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