Herbal Medicine quality and safety issues
- a primer for health professionals

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Expectations of all medicines

EFFECTIVE

SAFE

QUALITY
Medicine

- Environment
- Age
- Plant part
- Chemotype
- Species

Plant

Stability
- Formulation
- Extraction
- Post-harvest
- Harvest

Extraction

Formulation

Post-harvest

Harvest
The industry chain

- Grower / Collector
- Primary processor / Regional middlemen / Cooperative
- Middlemen
- Exporter
- Consumer country importer / Secondary processor
- Retailer / Practitioner
- Patient / Consumer
- Secondary processor / National retailer
- Consumer / Patient

After Booker A et al, *J Ethnopharmacology* 2012;140:624-33
The challenge of herbal quality control

Chemical complexity

Natural variability

CONSISTENT MEDICINE
Analytical Chemistry 101 vs Advanced Analytical Chemistry
Aspects of quality of medicines

• Authenticity
  - they are what they purport to be

• Consistency
  - they have a consistent composition

• Purity
  - they contain ONLY what they’re supposed to contain
Authenticity
Thyme from Morocco

Thymus sp.  Thymus vulgaris
Comparative chemistry

HPTLC

HPLC

Fluid extract

Authentic reference
There is something about Marigold...

Authentic reference

Extract
Don’t rely on common names!

- *Calendula officinalis*
- *Tagetes erecta*
## Certificate of Analysis

<table>
<thead>
<tr>
<th>Certificate of Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRODUCT NAME</strong></td>
</tr>
<tr>
<td><strong>LATIN NAME</strong></td>
</tr>
<tr>
<td><strong>PART OF USED</strong></td>
</tr>
<tr>
<td><strong>BATCH NO</strong></td>
</tr>
<tr>
<td><strong>BATCH SIZE</strong></td>
</tr>
<tr>
<td><strong>MANU DATE</strong></td>
</tr>
<tr>
<td><strong>SHELF LIFE</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>ITEM</strong></th>
<th><strong>SPECIFICATION</strong></th>
<th><strong>RESULT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Light Brown Yellow Fine Powder</td>
<td>Conforms</td>
</tr>
<tr>
<td>Extraction Solvent</td>
<td>100% Water</td>
<td>Conforms</td>
</tr>
<tr>
<td>Extract Ratio</td>
<td>4:1</td>
<td>Conforms</td>
</tr>
</tbody>
</table>
Skullcap (*Scutellaria lateriflora*)

- Native to N America
  - cultivated US, Chile, Mexico, Costa Rica, Australia
- Leaf rich in flavonoid glycosides
  - baicalin (~5%), dihydrobaicalin, lateriflorin
- King’s Dispensatory 1898:
  - ‘larger part of the drug sold... is derived from two other species...’
- AHP Monograph 2009:
  - substituted with *S. galericulata*, *S. alpina*, *S. incana*, *S. baicalensis*, *Teucrium* spp.
HPTLC on silica gel 60 F$_{254}$ plates
Mobile phase: tetrahydrofuran:toluene: formic acid:water (16:8:2:1 v/v)
Visualisation: Natural Products Reagent followed by polyethylene glycol 4000
Photographed under UV 366 nm

S. lateriflora (6 samples)
S. lateriflora (left)
Scutellaria sp. (4 samples)

Left to right:
Scutellaria sp.
S. baicalensis root
S. galericulata
Scutellaria ‘Bluebird’
S. baicalensis aerial
Scutellaria 'Bluebird' S32
S. lateriflora S2
S. lateriflora S37
S. galericulata S35
S. barbata S43
S. altissima S34
S. baicalensis root S23
S. baicalensis leaf S33
Scutellaria sp. S5
Concentration mg/ml

Scutellarin
Baicalin
Baicalein
Chrysin
Looking for *Teucrium* spp.

- No *Teucrium* found in *Scutellaria* samples
- *T. chamaedrys* contained teucrioside
  - no verbascoside
  - no flavonoids typical of *Scutellaria*
Saffron - the world’s most precious botanical commodity

- Styles and stigmas from *Crocus sativus*
- Retails for $10-30 per gram
- 100-150 flowers needed for 1 g
- Spice
- Anti-cancer, anti-depressant activity
70% EtOH extracts from (l-r): Iran, Tasmania, Kashmir
Fake saffron

Authentic saffron
Variability

![Graph showing variability in percentage extracted with different percentages of ethanol.]
Natural variability

8 wild SJW population in Montana and N California

- 417-fold difference in hypericin level
- 445-fold difference in pseudohypericin level
- Relative ratio varied but correlated

Sirvent et al. Economic Botany 2002
Passiflora incarnata

- Some material grown in Australia have atypical C-glycosyl flavone profiles.

Wohlmuth et al. Biol Pharm Bull 2010

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
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</thead>
<tbody>
<tr>
<td>vitexin (1)</td>
<td>H</td>
<td>OH</td>
<td>glucosyl</td>
</tr>
<tr>
<td>isovitexin (2)</td>
<td>glucosyl</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>schaftoside (3)</td>
<td>glucosyl</td>
<td>OH</td>
<td>α-L-arabinopyranosyl</td>
</tr>
<tr>
<td>isoschaftoside (4)</td>
<td>α-L-arabinopyranosyl</td>
<td>OH</td>
<td>glucosyl</td>
</tr>
<tr>
<td>swertisin (5)</td>
<td>glucosyl</td>
<td>OCH₃</td>
<td>H</td>
</tr>
</tbody>
</table>

Six Australian samples (1-6) and two typical samples. (C=chlorogenic acid, H=hyperoside, R=rutin.)
**Passiflora incarnata** chemotypes

- Much (but not all) material grown in Australia is of swertisin chemotype
- Typical material is of shaftoside/isovitexin chemotype

Wohlmuth *et al.* Biol Pharm Bull 2010
Extraction solvent matters!

Rosmarinic acid from Melissa
- extraction efficiency of different solvents
Non-alcoholic liquid extract

Sorbate:
E200 Sorbic acid
E201 Sodium sorbate
E202 Potassium sorbate

60% EtOH
Alkylamides in Echinacea preparations

60% EtOH
0% EtOH
Purity

Free aglycones (calculated as quercetin):
pre-hydrolysis as percentage of post-hydrolysis

Genistein

Quercetin
Kaempferol
Isoflavone
Q+K+I
Bilberry Adulteration Using the Food Dye Amaranth

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Table 1. Comparison of Anthocyanin Content of the Two Bilberry Extracts Using Different Analysis Methodsa

<table>
<thead>
<tr>
<th>sample</th>
<th>% anthocyanins (w/w)</th>
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<tbody>
<tr>
<td></td>
<td>BP</td>
</tr>
<tr>
<td>unadulterated bilberry</td>
<td>24</td>
</tr>
<tr>
<td>adulterated bilberry</td>
<td>24</td>
</tr>
</tbody>
</table>

a BP, British Pharmacopoeia single-wavelength spectroscopic method (3); HPLC, high-performance liquid chromatography method; INA, INA dual-wavelength spectroscopic method (4).

Absorbance Change (587 - 410 nm) vs pH

- Bilberry extract
- Adulterated extract
- Amaranth
Composition of *Ginkgo Biloba* products

2 October 2009

*Ginkgo biloba* (the Maidenhair tree) is a deciduous tree native to China, Korea and Japan. Ginkgo leaves and seeds have a long history of use in traditional Chinese medicine for a range of conditions. Ginkgo is one of the most widely used herbal medicines in Europe and the United States of America.

There are currently more than 400 products that contain Ginkgo on the Australian Register of Therapeutic Goods (ARTG). These are listed medicines and are commonly indicated for stimulating blood circulation.

The TGA has recently conducted testing on twenty-two (22) batches of medicines, covering twenty (20) products, and a number of associated Ginkgo extract raw materials. The testing focused on determining the quality of the Ginkgo extract used in the formulation of the medicines.

In some of the samples tested, elevated levels of quercetin and rutin were noted. These are naturally occurring components found in Ginkgo and many other plants. Variation in the content of these components may occur due to natural variation in the plants or the processing and storage of the herbal material.

The TGA is currently working with its expert advisory committees and the complementary medicine industry associations to refine the quality standards for Ginkgo extracts used in medicines available in Australia.
Ginkgo biloba adulteration

- **USP, EP/BP**
  - 22-27% flavonoids, expressed as flavonol glycosides, but quantified after hydrolysis
  - specified aglycone ratios:
    - kaempferol : quercetin 0.8-1.2
    - isorhamnetin : quercetin ≥0.1
- **Does not allow for detection of adulteration with aglycones!**

Solution!

Assay aglycones before and after hydrolysis:

\[ A_{\text{glycosides}} = A_{\text{post}} - A_{\text{pre}} \]
Ginkgo biloba adulteration

- Ginkgo leaf (5) contained no free aglycones
- 8 ginkgo products tested
Ginkgo biloba adulteration

- Current USP method overestimated flavonoid glycoside content by 29-41%.

Free aglycones (calculated as quercetin):
pre-hydrolysis as percentage of post-hydrolysis
Ginkgo biloba adulterant?

- **Styphnolobium japonicum**
  - genistein
  - quercetin and kaempferol glycosides
  - previously reported as a suspected adulterant of ginkgo extracts

Heavy metal contamination

- Heavy metals may be intentionally added to Indian and Chinese remedies
- Some plants accumulate heavy metals from the soil (e.g. Hypericum, Salix – cadmium) (Wenzig & Bauer, in Houghton & Mukherjee, Evaluation of Herbal Medicinal Products 2009)
- 3.5% of 317 batches of dried herbs delivered to German TCM hospital had heavy metal content exceeding legal limits (Melchart, Alt Ther Health Med 2001)
Pharmaceuticals in traditional Chinese medicines

In Taiwan, 24% of 2600 TCM preparations contained at least one drug compound
(Huang, J Clin Pharmacol 1997)

In the US, 10% of 500+ Chinese patent medicines contained undeclared drugs and/or toxic levels of heavy metals
(Au, Bull Env Contam Toxicol 2000)
Herbal Viagra products...
Herbal Viagra - indeed!

THIOHYDROXYHOMOSILDENAFIL

Use of a Hydrolytic Procedure and Spectrometric Methods in the Structure Elucidation of a Thiocarbonyl Analogue of Sildenafil Detected as an Adulterant in an Over-the-Counter Herbal Aphrodisiac

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Tea tree oil

Oil of *Melaleuca*, terpinen-4-ol type

ISO 4730, Australian Standard 2782:

- $\alpha$-pinene: 1-6%
- Sabinene: tr-3.5%
- $\alpha$-terpinene: 5-13%
- Limonene: 0.5-1.5%
- $p$-cymene: 0.5-8%
- 1,8-cineole: tr-15%
- $\gamma$-terpinene: 10-28%
- Terpinolene: 1.5-5%
- **Terpinen-4-ol**: 30-48%
- $\alpha$-terpineol: 1.5-8%
- + 4 others
Tea tree oil - adulteration issues

- Terpinen-4-ol content >40% attracts premium
- Adulteration with t-4-ol from other sources:
  - pine bark
  - marjoram
  - synthetic
- T-4-ol chiral ratio (+/-) should be ~2.1
- Synthetic oil
  - lacks sesquiterpenes
  - short-chain hydrocarbons
Tea tree oil - safety concerns

- Methyl eugenol
  - $<0.01-0.06\%$ (mean 0.02%)
  - $10^6$ times lower than carcinogenic dose in rats

- Contact allergy

- Sensitising potential linked to oxidation products
  - increased $p$-cymene
  - peroxides, endoperoxides and epoxides
  - protect from oxygen, light, heat

Southwell et al. Flavour & Fragrance 2011
Conclusions

• Quality assurance of natural products presents unique challenges

• Rigorous quality assurance must be maintained to ensure the quality, safety and effectiveness of herbal medicines
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