Medicinal Plants & Dietary Supplements & Botanical Standardization

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Challenges in Medicinal Plants, Plants and Plant Products Used at Dietary Supplements and Botanical Standardization

• The plethora of products
• Range from simple single botanical ingredients, yet with multiple bioactives and/or unknown or still to be agreed upon bioactives
• Multiple ingredient products compounding the dilemma and challenges yet requiring a rigorous approach to traceability/standardization
• Practical and programmatic: high tech vs low tech approaches
Plant identification is challenging
ATTACHMENT A: REGULATORY APPROACHES FOR MARKETING BOTANICAL DRUG PRODUCTS

Diagram:

1. Botanical Product
   - Applied to body for cleansing, beautifying, or altering appearance
   - What is the intended use?
      - Consumed for its taste, aroma, or nutritive value
      - To affect structure or function of body

2. To diagnose, cure, mitigate, or treat disease
   - To prevent disease
     - Yes: Conventional Food or Dietary Supplement
       - Authorized health claim?
         - Yes
           - Yes: Drug
             - Meets dietary supplement definition in 21 U.S.C. 321 (ff)?
               - No: Dietary supplements
               - Yes: Structure/function claims comply with the requirement
         - No: Drug
   - No: Drug

www.fda.gov
Botanicals & Dietary Supplements: The Concerns

• Hundreds of botanicals from all over the world are sold in the US market.
• Lack of strict regulation from the government.
• Ample traditional medical practitioners in other countries, but a distinct shortage of scientists with knowledge of traditional use, botanicals authentication, natural products chemistry.
• Lack of reference materials.
• Lack of quality control standard and pure botanical reference standards.
• Lack of validated and universal analytical methods although we have AOAC, USP, INA, others in process.
• Lack of validated certification program although we have NSF and other NGO and for-profit testing services and FDA.
• Adulteration of botanical products (purposeful or inadvertently)
Quality Control: A Multi-Prong Approach

- Botanical authentication (use of botanical, chemical and genetic fingerprinting)
- Pre-selection of criteria desired in a chemical screen
- Confirmation of an acceptable chemical profile(s) both at the start and termination of planned study.
- Proper storage and retention of reference materials and product(s) used in study
Botanical Standardization is Core to A QC Program and for the Study and Reporting of All Health Studies

- QC is chemically testing your botanicals plus more
- QA and QC are key parts of a real and dynamic system that ensures traceability and product definition
- Ensures correct plant species
- Ensures a QA program in collection, production, and processing;
- Adulteration is a problem-purposeful & inadvertent;
- Adulteration problems have led to interest in cultivation rather than collection and have been largely responsible for the many news stories about herbal toxicities;
- Microbiological contamination
- Natural product(s) profiles- the natural products present
- Physicochemical properties
- Must conduct independent analysis on herbal products to be used (not rely on nor report on their own label information)
Minimum Guidelines for Quality Specifications for Plant Materials and Preparations*

For fresh, dried and processed plant materials:

• Name of plant(s) (latin, common, genus, species, and family);
• Plant part(s) used;
• Time and method of collections, preliminary preparation and drying, method of processing;
• Description and habitat of plant; wild or cultivated; listing any chemical inputs;
• Characterization of compounds, which may also be the BAC, including structural formulae.

Quality Specifications:

• Authenticity;
• Purity
• Assay
• Packaging, labelling and storage

Authenticity:
- Macroscopic, microscopic and ‘sensory characteristics’ plus voucher specimens and/or product lots.
- How was authenticity achieved? Fingerprinting: Chemical, botanical, genetic? Description of tests.

Purity:
- Limits of foreign matter (organic and mineral); ash determinaton

Assay:
- A physical, chemical or biological assay of known or active fractions should be described along with an acceptable range for the assay results.

Packaging, Labelling and Storage:
- Keep records and track of these
- Keep extra reference and study materials for future inquiries
Minimum Guidelines for Quality Specifications Needed for the Medicinal Preparations of Plant Materials for Evaluating the Safety and Efficacy of Herbal Medicines*

“Among the medicinal preparations, the herbal products fall into a wide range of preparations from powders, granules, pills, extracts, tablets, injections, more.

Traditional powders and pills are made of powdered plant materials, tablets, granules, ointments and extracts; injections are made of purified extracts or pure active constituents isolated from the plant. Other preparations contain both powdered plant materials and extracts.”

*WHO, Research Guidelines for Evaluating Safety and Efficacy of Herbal Medicines, 1993
Minimum Guidelines for Quality Specifications Needed for the Medicinal Preparations of Plant Materials for Evaluating the Safety and Efficacy of Herbal Medicines, con’t

• Name in Latin, English and native languages
• Formula including the name of each ingredient and quantities used for 1,000g or 1,000ml of the product. A quantity may be given as a range corresponding to a definite quantity of assayed active constituent. Any excipient used should be specified.
• Method of preparation to make the 1,000g or 1,000ml of product. Description of the method should include details of any process, solvent, time, temperature of an extraction and concentration, and the process used to reduce the level of microbial contamination.
• The active constituents, as far as they are known, should be stated, their structural formulae given. Any chemical and pharmacological incompatibility should be cited.
Minimum Guidelines for Quality Specifications Needed for the Medicinal Preparations of Plant Materials for Evaluating the Safety and Efficacy of Herbal Medicines, from WHO con’t

**Authenticity:** same as with background information on fresh, dried, and processed materials, but now also to add:
- physical or chemical identification tests described and TLC and/or other procedures used in characterizing compounds
- drawing or photograph of the chromatograms should be included.
- for compound preparations, the most important ingredients should be fingerprinted.

**Purity:** limit tests for heavy metals in extracts and tests for freedom from methanol in alcoholic preparations should be specified. Limit tests for contaminants such as pesticides.

**Assay:** Content of BAC and those that influence the efficacy of the product should be determined and found to be in an acceptable range. For herbal mixtures, the most characterizing compounds possible should be assayed.
Tests related to the form of the preparation for both non-clinical and clinical tests should follow the available regulatory requirements of your country.

**Packaging, labelling and storage:** all recorded and same as with fresh, dried, processed materials.
Quality Control (QC) Goes from ‘Bush’ or Source of Raw Materials to Final Product

Which is Pygeum (Prunus africanaum)?
Ginseng

• One of the most important herbs in eastern herbal medicine.
• Used for thousands of years to combat psychophysical tiredness and as a tonic.
• Pharmacological activity: free radical scavenging activity, immunological effects, action on the central nerve system & metabolic activity, much more.
• The saponins (ginsenosides) are the main components and marker compounds inside (see U of Illinois’s research and others.).
HPLC profiles of Asian and American Ginseng
Conclusions: Of the four commercial samples sold as root extracts only one sample tested appears to be from the roots.
Quality of Commercial Ginseng Products

- Six ginseng (Panax ginseng) were selected randomly for testing; all claimed as root or root extract. Samples were repackaged so all testing was blind.
- Four commercial sources reported the amount of ginsenosides; three met their claims, one failed containing only 50% of the reported minimum and actually appeared to be a leaf extract rather than a root extract.
- Two were labeled as Ginseng powder, one source appeared to be a real ginseng main root powder and contained 1.4% ginsenosides; but the other one contained 0% ginsenosides inside.
- This rapid assessment suggested a 66% approval rating.

![Bar graph showing the comparison between claimed and tested ginsenoside content in various ginseng products.]

- **Claimed ginsenosides per serve (mg)**
- **Tested ginsenosides per serve (mg)**
Goldenseal

- Latin name: *Hydrastis canadensis* L.
- Origin: North America
- Part used: Dried Rhizome and roots
- Pharmacological activity: antibacterial and anti-inflammatory
- Uses: Treat nasal congestion, mouth sores, eye and ear infections, and purported to enhance immune system,
- Active components: isoquinoline alkaloids with hydrastine (1.5-4%) and berberine (0.5-6%), canandine.
Quality of Goldenseal Raw Materials

Goldenseal root powder (6 samples): all passed. Hydrastine ranged in the content from 2.25-2.86% and berberine ranged from 2.61% to 3.21%. In contrast: Goldenseal standardized extracts (10 samples): while all the samples contained acceptable levels of berberine, only 50% of the samples contained hydrastine.
Quality of Finished Products with Goldenseal

- Total of five products were tested, all products were removed and repackaged for blind testing
- The commercial products included 3 Goldenseal/Echinacea combinations and 2 Goldenseal root extracts
- Dosage range reported on label: 1.25-57 mg alkaloids per serving
- One product had very low alkaloids contents compared with claimed value (failed).
- Another product contained only berberine, no hydrastine (failed).
- A third product contained 27.1 mg total alkaloids but their label cited 10 mg hydrastine (while the natural product level exceeded the minimum, it failed our test as not being reflective of the actual content).
- Two passed. This rapid assessment suggests that 40% approval rating.
Latin name: *Echinacea angustifolia*, *E. purpurea*, and *E. pallida*

The roots of *Echinacea angustofolia*, *purpurea* and *pallida* are used as herbal medicine. The tops of *E. purpurea* is also used as herbal medicine.

Immunostimulants.

One of the best selling herbs in United States and Europe.

Caffeic acid derived compounds, unsaturated aliphatic compounds and polysaccharides are major compounds inside.
Major phenolic compounds in Echinacea

- Cichoric Acid
- Chlorogenic Acid
- Caftaric Acid
- Echinacoside
HPLC Profiles of Different Species of Echinacea

E. purpurea

E. angustifolia

E. pallida

E. paradoxa var. neglecta
HPLC profile of a questioned *Echinacea angustifolia* sample

Is this *E. Angustifolia*?
RAPD profiles generated with primers OPH-19 (A), OPH-13 (B), OPC-2 (C), and OPG-9 (D). Lanes M: pGEM ladder, 1-4: *E. angustifolia*, 5-8: *E. pallida*, 9-12 *E. purpurea*
Work of Wolf et al. (1999) demonstrating the application of RAPD analysis for the identification of *Echinacea* species in mixtures of drug or root material.
Identification of *P. integrifolium* in mixed tissue samples of bulked tissue of *E. angustifolia* and *P. integrifolium*. Leaf tissue of 8 individuals of each species was bulked and mixed in 4 ratios prior to DNA extraction. DNA samples were amplified using primer O-13, and products were separated on a 5% acrylamide gel.

Lane 1,2 - *P. integrifolium* individuals, **Lane 3 - 1:1 E. angustifolia:*P. integrifolium, Lane 4 - 3:1, Lane 5 - 9:1, Lane 6 - 99:1, Lane 7,8 - *E. angustifolia* individuals
Promise and Challenges with PCR also include:

- PCR methods have great potential for use in the identification of botanicals, in the detection of more than one herb, and in the detection of many types of adulterants;
- Challenges include working with genetic systems not well characterized; where there can be taxonomical confusion even among botanists; where reference materials do not lend themselves to historical taxonomical evaluation; and where many plants are mixed and sold together;
- Still faces major issues: (1) purity and adulteration; (2) ensuring standardized botanical products/extracts for mode of action studies and clinical evaluation; and (3) is material of pharmacological value even if pure? (e.g. NPs low or absent in a plant)
- Even with major advances in PCR methodologies, QA for herbal medicines will likely need to incorporate more than one screen to achieve such wide goals; suggesting that other chemical and biological assays will remain critical in nutritional/medical studies. PCR systems will be a part of QC and botanical standardization.
Ginkgo (Ginkgo biloba L.)

- Latin name: Ginkgo Biloba L.
- Origin: Northern China
- Part used in herbal medicine: leaves
- Pharmacological activity: Antiaging, Alzheimer's disease, asthma, depression, attention deficit disorder, blood clots, circulatory insufficiency, memory loss, respiratory disease, senility, stress, stroke, tinnitus and vascular disease.
- Active components: flavonoid glycosides and terpene lactones.
HPLC profiles of flavonoid glycosides in Ginkgo

Spiked with quercetin
Arctic root

Latin name: *Rhodiola Rosea*

Origin: Eastern Europe and Asia

Part as medicine: root

A plant adaptogen like ginseng, decreasing depression, enhancing work performance, eliminating fatigue, and preventing high altitude sickness.

The major chemical inside are salidroside, rosavins and flavonoid.
Structures of Marker compounds in \textit{Rhodiola rosea}

- Salidroside
- Rosavin
- Rosin
- Rosavin
HPLC profile of *Rhodiola Rosea*

- No rosavins
- No rosavins
- No rosavins
- No rosavins
Soy

- Soybeans are among the world’s most valuable crops.
- The isoflavones, daidzein, genistein and glycine, and their glycosides are the major phenolic compounds in soy.
- Soy isoflavones have exhibited bioactivity to lower the risk of cancer, heart disease, osteoporosis, and the relief of menopause symptoms.
- In addition to breast cancer, soybean isoflavones may help reduce the risk of several types of cancer, including lung, colon and rectal cancer. One cancer that is receiving a lot of attention in relation to isoflavones is prostate cancer. Isoflavones have been shown in animal models to slow the growth of and to delay the development of pre-cancerous prostate lesions and prostate tumors.
Structure of Major Isoflavones in Soy

Structures of the Target Compounds:

Daidzein (C_{15}H_{10}O_{4}, MW: 254)

Genistein (C_{15}H_{12}O_{5}, MW: 270)

Glycitein (C_{16}H_{12}O_{5}, MW: 284)

Daidzin (C_{21}H_{20}O_{9}, MW: 416)

Genistin (C_{21}H_{22}O_{10}, MW: 432)

Glycitin (C_{22}H_{22}O_{11}, MW: 446)
HPLC profile of different Soy extracts: Pure Samples Vs. Spiked

Real pure soy extract
  Daidzin: Genistin about 1:1

Extra genistin

Soy germ extract

?
Schisandra (Weiweizi)

- Latin name: *Schisandra Chinensis*
- Origin: Northern China
- Part as herbal medicine: fruits
- Pharmacological activity: tonic, CNS stimulating activity, anti-hepatotoxic, antioxidant.
- A drug to treat nonicteric hepatitis
- Active components: containing over 30 lignoid-type compounds. The major one is Schisandrol A.
Structure of major lignans in Schisandra

Schisandrin A

Schidandrin B

Schisandrol A

Schidandrol B
HPLC Profile of Schisandra Berry and Extracts

• Total 1 berry sample and 10 powder extracts (9 :1 extract or 9% lignans) were tested.
• Only the berry and two extracts match the profile of schisandra.
• This rapid assessment suggests a 28% approval rating.
Tribulus

*Tribulus terrestris* is a weed that grows around the world and is commonly known as “Puncture Vine” or Caltrop fruit.

• Used to treat a wide variety of health problems including loss of libido (sex drive), impotence, infertility, edema, liver, kidney and heart problems.

• The major chemicals in tribulus include saponins with protodioscin as the dominated component.
HPLC profiles of *Tribulus* from different regions
Red Clover
*(Trifolium pratense)*

**Chemical Profile:** Isoflavones, Flavones and Saponins

**Bioactivity:** Estrogenic Activity-for Postmenopausal symptoms and to protect bone
UV Chromatograms of Red Clover Leaf and 3 typical Commercial products (Top=original, all others are commercial samples)

Red Clover Leaf (NY)

Nature’s Way Inc.
Facts: Red Clover Arial Part Powder

Vitamin Shoppe
Facts: Red Clover Extract (Herb) and Flower Powder

Solgar Vitamin and Herb
Facts: Red Clover Extract (Leaf) and Leaf Powder
Example 1: Anthocyanin-containing herbs

1. Bilberry
2. Blueberry
3. Red grape
4. Red cabbage
5. Black current
6. Elderberry
7. Cornflower
8. Purple basil

Basic structures of anthocyanins

- $R_1 = R_2 = H$, Pelargonidin
- $R_1 = OH, R_2 = H$, Cyanidin
- $R_1 = OCH_3, R_2 = H$, Peonidin
- $R_1 = R_2 = OH$, Delphinidin
- $R_1 = OCH_3, R_2 = CH$, Petunidin
- $R_1 = R_1 = OCH_3$, Malvidin
Total anthocyanidins in bilberry products by colorimetric method (Low in the contents of active components)
Quality of bilberry products (wrong plant materials)

Samples 1-5

Sample 6-7
Elderberry
Quality Control Issues Facing Botanicals Used in the Natural Products Industry:

• Several popular herbal dietary supplements were comparatively screened for quality relative to their natural products content.

• The issue of quality control is important. Many botanicals are “quality challenged”: natural products may vary from stated label claim (if there is one); another plant part or plant species is used; adulteration is more common than acknowledged; some products can be spiked with synthetic chemicals or compounds from other botanicals.

• Reference materials for these botanicals needs to be available through governmental and research institutes.

• Universal analytical method should continued to be developed and validated for the most popular botanicals, and in particular for those botanicals that are problematic from a safety perspective.

• Detailed genetic and chemical fingerprinting for every popular herb is needed. (www.nuanpp.org)
Ravintsara, *Cinnamom camphorum* (Malagasy Natural Product Label-Top); *Ravensara aromatica* (US Company-Middle) which despite its claim as a different essential oil shows the same oil profile (using MS) as the TOP oil indicating it is incorrectly sold; *Ravensara aromatica* (Bottom) is the real authenticated product.
A Commercial Bottle of *Ravensara aromatica* that should instead be sold as Ravintsara (*Cinnamomum camphora*). A real QC program would have precluded this problem!
Pesticides, Heavy Metals and Microbiological Contamination

- Pesticides are used today both in the field for insect and disease control, in storage to deter insects and rodents and other pests, and sometimes the plants are in a proximity where pesticide spray aimed at another target or building reaches the medicinal plant. Only those pesticides which are allowed by law in the targeted marketplace where the products are to be sold can be used.

- Both domestic and international imports subject to random testing- and there have been cases where botanicals have been found to contain illegal or excessive concentrations of a pesticide (e.g. Ginseng imported from China).

- Heavy metals
- Microbiological contamination
How Real is This?

- Surveyed 193 of the 230 requested on-lines medicines were received and analyzed.
- Lead, mercury, and arsenic have been detected in a substantial proportion of Indian-manufactured traditional Ayurvedic medicines. Metals may be present due to the practice of *rasa shastra* (combining herbs with metals, minerals, and gems).
- Whether toxic metals are present in both US- and Indian-manufactured Ayurvedic medicines.
- Among the metal-containing products, 95% were sold by US Web sites and 75% claimed Good Manufacturing Practices. All metal containing products exceeded 1 or more standards for acceptable daily intake of toxic metals.

Researchers conclude that 1/5 of both US-manufactured and Indian-manufactured Ayurvedic medicines purchased via the Internet contain detectable lead, mercury, or

*Saper et al. JAMA 300 (8): 915-923. August 27, 2008.*
Adulteration is a Major Problem: Shea Butter (*Vitellaria paradoxa* C.F. Gaertn.)

- Shea butter used widely in cosmetics, but also an edible oil, food product, and a carrier of essential oils and pharmaceuticals.
- With inexpensive fats: Coconut oil, palm/palm kernel oil
- Detected by FA composition:
  - Coconut/palm kernel oil rich in C12:0 and C14:0
  - Palm oil rich in C16:0
  - Shea nut butter rich in 18:0

*Melting point for pure shea butter should be 36-37°C deviations from this should indicate compromised purity.*
FA Composition of Adulterated shea butter - 45% coconut oil

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Sheanut</th>
<th>Sheanut + Coconut</th>
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<tr>
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<td>–</td>
<td>3.2</td>
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</table>
Quality Control and Assurance

• QA and QC are key parts of a real and dynamic system that ensures traceability and product definition.

• Quality assurance is the system of monitoring, inspection and auditing which justify the product has been made according to the protocols and standard operating procedures (SOPs).

• Quality control is the regulatory process through which we measure actual quality performance, comparing it with quality goals and acts on the difference.
General Quality Control Parameters

Step 1: Raw material authentication:
- Reference materials
- Plant parts
- Macroscopic and microscopic examination
- Thin layer chromatography
- Infrared Spectroscopy
- Near infrared spectroscopy
- Genotypic (DNA) analysis
Step 2: Chemical Analysis
Wet chemistry

• Moisture
• pH value
• Solubility
• Density
• Particle size
• Viscosity
• Ashes
• foreign matters
Step 3: Safety

• Heavy metals
• Aflatoxins
• Pesticides
• Toxic compounds such as aristolochic acid
• Radiation and adulteration

Health Highlights: Dec. 16, 2004
12/16/2004
Here are some of the latest health and medical news developments, compiled by the editors of HealthDay:

FDA Issues Warning on Pesticides Found in Ginseng
The U.S. Food and Drug Administration has issued a nationwide warning after having discovered residue of untested pesticides in ginseng imported by a New Jersey firm.

The FDA says U.S. marshals seized a quantity of ginseng from FCC Products Inc. of Livingston, N.J., and found residue from the chemicals procymidone and quintozene. And while the agency says the distribution was probably small, it issued the nationwide alert for anyone who may have bought the ginseng from the New Jersey company because the chemicals haven't been officially tested for how well they're tolerated by humans.

"A raw agricultural commodity or a processed food or feed is deemed to be unsafe and adulterated, and subject to FDA enforcement action, if a pesticide chemical residue for which no tolerance has been set is present in food," the FDA said in a statement.

Ginseng is an herbal root, usually from Asia, that is often used to increase energy levels. It is classified as a food supplement and not usually subject to FDA regulations.
Microbiological tests

- Total Bacteria testing
- Mold and Yeast testing
- \textit{E. coli} testing
- \textit{Salmonella} testing
Additional quality control Considerations in Herbal Extracts and Products

- Cut size of starting herbs
- Ratio of the herbs to the extraction solvent
- Extraction time
- Temperature load during extraction, evaporation, drying and storage
- Microbial decontamination
Types of Standardized Extracts

- Extracts standardized to an active component (Yohimbe)
- Extracts standardized to a group of bioactive components (tea polyphenol extract)
- Extracts standardized to a few groups of bioactive components (ginkgo extract)
- Extracts standardized to one or two types of compounds (Standardized saponin extract of Tribulus)
- Extracts standardized to marker compounds (Siberian ginseng)
Analytical Instrumentals used for standardization and fingerprinting of botanical extracts

- UV/Vis spectroscopy
- HPLC
- LC/MS
- GC and GC/MS
- Electrophoresis