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The Role of Quality Assurance and Standardization in the Safety of Botanical Dietary Supplements

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Introduction

The importance of complementary and alternative therapies such as botanical dietary supplements continues to increase throughout the world (1). The World Health Organization (WHO) has estimated that the majority of people in developing countries depend on traditional and herbal medicines as their primary source of health care (2). In the United States, 42% of the population have reported using complementary and alternative medicines, especially botanical dietary supplements (3), costing an estimated \$5.1 billion per year (4). The marketing and use of dietary supplements has grown rapidly in the United States following the passage of the 'Dietary Supplement and Health Education Act' in 1994, which exempts dietary supplements from regulation as drugs providing that they are not marketed for the diagnosis, treatment, cure, or prevention of disease (5).

Although the use of botanical dietary supplements has increased substantially during the last two decades, evidence for their efficacy and safety has not been well documented. In the United States for example, botanical dietary supplements are currently exempt from good manufacturing practice, do not require proof of efficacy, and do not require pre-marketing approval by the FDA unless drug-like claims are made. The safety of botanical dietary supplements is the responsibility of the manufacturers, and the role of the FDA in safety assurance is limited to post-marketing monitoring of adverse effects. Since no disease treatment or prevention benefits may be claimed for dietary supplements, they are exempt from FDA regulation, and efficacy studies for these products are relatively rare.

Therefore, the safety and efficacy of most botanical dietary supplements lack documentation, which concerns many health care providers and consumers. Possible problems with botanical dietary supplements include contamination with pesticides, herbicides and heavy metals, contamination or adulteration with pharmacologically active medications, use of the incorrect part of the plants (for example, leaves instead of roots), and even misidentification of the plant species incorporated into the product. Since no toxicology studies are required for botanical dietary supplements, there is also concern that interaction might occur between botanicals and conventional pharmaceuticals or that metabolic activation of constituents in the botanical

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dietary supplement might result in the formation of toxic metabolites. The possibility of overdose is also an issue, since studies to establish maximum tolerated dosages and safe long-term chronic dosages are not required and are rarely carried out.

Since botanical dietary supplements may be marketed until proven unsafe through the documentation of adverse effects, the safety of these products is determined primarily through self-regulation by manufacturers. Since consumers expect a consistent and safe product, the agricultural and herbal industries should work together to produce safe products of reproducible quality using basic principles of botany, chemistry and pharmacology. This review addresses basic safety issues concerning botanical dietary supplements. Specific problems that have occurred with respect to safety are described, and solutions to these safety issues are proposed.

Acquisition of plant material

The production of safe botanical dietary supplements of high quality begins with plants of the correct species. Botanicals intended for use in dietary supplements should be cultivated and harvested using good agricultural practices, and field collected material should be acquired using good collection practices. Each batch of plants used for the production of a dietary supplement should be identified using taxonomic examination (macroscopic and/or microscopic) and/or biochemical or chemical tests. Milled plant material may be identified microscopically. For example, to ensure the quality of rhizomes of *Cimicifuga racemosa* (L.) Nutt. (*Actaea racemosa* L.) for clinical trials of safety and efficacy, Fong, *et al.* (6) used good field collection practice in the mountains of eastern North America and identified and validated the specimens by macroscopic, microscopic and DNA analysis (7,8). DNA may be isolated from intact or milled plants and analyzed using PCR techniques such as RAPD (randomly amplified polymorphic DNA) with comparison to authentic material (7,9,10). Alternatively, immunoassays may be used for identification based on the detection of species-specific proteins (9). In addition, botanically authenticated voucher specimens should be preserved for future reference.

If plant extracts are used in the preparation of botanical dietary supplements, then these should be purchased from suppliers who provide proof of taxonomic or genetic identification of the original plant material. However, when taxonomic or genetic analysis is not possible such as with plant extracts, then the processed material should be examined chemically with comparison to reference standards as an alternative form of quality assurance and identification. Such chemical evaluation might consist of high performance liquid chromatography (HPLC) with UV absorbance detection, HPLC with evaporative light scattering, or HPLC-mass spectrometry (LC-MS). Then, the plants used in the production of the extracts may be identified using the chromatographic data through the detection of species-specific marker compounds. This approach can include detection of compounds indicative of contaminating plants as well as of the expected species.

Human toxicity resulting from the misidentification of plant material used in the production of botanical dietary supplements has been reported. In a well documented example, the Center for Food Safety and Applied Nutrition of the US FDA published the case reports of two women suffering from atrioventricular block after ingesting botanical dietary supplements of the same brand-name and lot number (11). Neither subject had any history of heart disease. Because the cardiac symptoms were suggestive of digitalis toxicity, serum samples were tested using an immunoassay and found to be positive for digoxin. Next, a botanical dietary supplement used by both subjects, which was a combination of 14 herbal ingredients, tested positive for cardiac glycosides. Subsequently, samples of the ingredients used to prepare the supplement were obtained from distributors, and the plant material labeled as “plantain” was found to contain cardiac glycosides. Based on the identification of the cardiac glycosides lanatoside A and

lanatoside C in this material using LC-MS and microscopic anatomical examination, the plant was identified as *Digitalis lanata* instead of “plantain”. All 115 dietary supplements that had been produced using the plant material from this lot were recalled.

The substitution of related species for the botanical indicated on the label of a botanical dietary supplement, perhaps as a result of misidentification or because of confusion due to similar common nomenclature, can be prevented if samples of the botanical material are examined macroscopically or microscopically for taxonomic identification prior to processing into the finished dietary supplement. Alternatively, extracts of the plant material or the finished product can be analyzed by chromatographic techniques for the presence of characteristic compounds or profiles of signature compounds that confirm the identity of the appropriate species or the incorrect related species.

For example, American ginseng is prepared from the roots of *Panax quinquefolius* L., but it might be mixed with or replaced by the related Asian species *Panax ginseng* C.A. Meyer or the unrelated Siberian ginseng *Eleutherococcus senticosus* Maxim. Historically, only products prepared from the roots of the *Panax* species were considered ginseng, but the common name ginseng is sometimes used today to describe herbal products made from *Eleutherococcus senticosus*, i.e. “Siberian ginseng,” as well. Therefore, the substitution of one species for another might occur inadvertently. Even if all taxonomic and genetic indicators have been lost during the preparation of extracts, these ginseng species can be differentiated by the detection of characteristic ginsenosides from the *Panax* species or eleutherosides from Siberian ginseng (12,13).

The *Panax* species contain ginsenosides, which are triterpene saponins associated with the pharmacological activity of ginseng (14). In contrast, *Eleutherococcus senticosus* contains no ginsenosides but instead contains eleutherosides. In addition to the presence of ginsenosides which are unique to *Panax*, the relative amounts of ginsenosides may also be used to differentiate between *Panax* species. For example, American ginseng has little or no ginsenoside Rf, but does contain 24 (R)-pseudoginsenoside F₁₁ which is absent in Asian ginseng (see Figure 1). Further, the former has a lower ratio of ginsenoside Rg₁ to Rb₁ than the latter species (13,15). Therefore, chromatographic analysis, usually using mass spectrometry, tandem mass spectrometry or evaporative light scattering detection, may be used to determine which species of ginseng has been used in a dietary supplement. Furthermore, the levels of ginsenosides or eleutherosides may be measured for the standardization of ginseng products.

The World Health Organization Programme on Traditional Medicine has published guidelines for good agriculture and collection practices in the acquisition of quality botanicals for research (16). Furthermore, the National Center for Complementary and Alternative Medicine of the US National Institutes of Health has established an interim guidance on product quality for grant applicants (17). The implementation of these guidelines by producers of botanical dietary supplements will help assure that the correct plant material is utilized in the production of botanical dietary supplements.

Finally, the appropriate parts of the plants should be used for the production of botanical dietary supplements. For example, if roots are to be used in the supplement, then the aerial parts of the plant such as leaves and stems should be excluded. As in edible plants, only certain parts of the plant might be safe for human consumption. An example is the tomato (*Solanum lycopersicum* L.) from which the ripe fruit is safe for consumption as a food or for use in the preparation of lycopene-rich dietary supplements. However, other plant parts of *S. lycopersicum* such as the leaves or the unripe fruits can contain toxic levels of the tomato

glycoalkaloids α -tomatine and dehydrotomatine (18) and should be excluded from the preparation of dietary supplements.

Contamination of botanical dietary supplements

Plants intended for use in botanical dietary supplements should be cultivated using good agricultural practice. This approach provides quality assurance by helping to prevent microbial, heavy metal, herbicide, and pesticide contamination and by excluding weeds and insects. If wild plant specimens are collected or if the plant material or extracts are purchased from suppliers without assurance of good agricultural practice, then they should be assayed for levels of pesticides, herbicides, heavy metals, and microbes.

The measurement of botanical dietary supplements for heavy metals is routine and usually utilizes techniques such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry. For example, Grippo *et al.* (19) used inductively coupled plasma mass spectrometry to analyze 27 botanical dietary supplements for 47 metals. All the supplements contained ephedra (*Ephedra sinica* Stapf) or ephedra in combination with black cohosh, Echinacea (*Echinacea purpurea* (L.) Muench), goldenseal (*Hydrastis canadensis* L.), kava (*Piper methysticum* Forster f.), milk thistle (*Silybum marianum* (L.) Gaertner), valerian (*Valeriana officinalis* L.), or saw palmetto (*Serenoa repens* (Bartram) Small). All 47 metals, which included lead, mercury and strontium, were within safe limits for daily consumption as directed by the producers. In another study by Raman *et al.* (20), botanical dietary supplements from commercial sources containing echinacea, garlic (*Allium sativum* L.), ginkgo (*Ginkgo biloba* L.), *Panax ginseng* C.A. Meyer, grape seed extract (*Vitis vinifera* L.), kava, saw palmetto, or St. John's wort (*Hypericum perforatum* L.) were analyzed for lead, mercury, cadmium, arsenic, uranium, chromium, vanadium, copper, zinc, molybdenum, palladium, tin, antimony, thallium, and tungsten using inductively coupled plasma mass spectrometry. No mercury was detected, and all other metals were within acceptable levels. Such analyses for heavy metals should be routine quality assurance practices by producers of botanical dietary supplements.

Assays for microbial content should be carried out as part of routine quality assurance of botanical dietary supplements. One outcome of botanical contamination by certain molds can be the formation of mycotoxins, which are toxic fungal secondary metabolites. Mycotoxins can be carcinogenic, teratogenic, immunogenic, and neurotoxic. Assays for mycotoxins in botanical dietary supplements have been reported and applied to products containing or derived from roots and rhizomes such as ginseng and ginger root. For example, Trucksess *et al.*, (21) developed assays for aflatoxin and ochratoxin A based on immunoaffinity chromatography followed by HPLC with fluorescence detection. A similar assay for aflatoxin B1 was reported by Arranz *et al.* (22) and utilized immunoaffinity extraction followed by HPLC with post-column derivatization and fluorescence detection.

The analysis of pesticide and herbicide residues in botanicals used in the preparation of dietary supplements should be a routine quality assurance step to help ensure human health. This practice should become routine as it is for fruits and vegetables entering the food supply. In addition, plant material containing excessive levels of potentially harmful pesticides should be excluded from botanical dietary supplements. To facilitate these tests, numerous chromatography-based assays have been developed for the quantitative analysis of pesticides and herbicides in botanicals. These assays are usually based on gas chromatography with either flame ionization detection, electron capture detection, or mass spectrometric detection. These assays have been standardized and are available from contract laboratories. The National Center for Complementary and Alternative Medicine of the US National Institutes of Health requires that grant recipients planning human studies using botanical dietary supplement

provide proof of analysis for pesticide residues as well as for heavy metals and microbiological contamination (17). As an example of the implementation of this policy, the UIC/NIH Center for Botanical Dietary Supplements Research, which is carrying out phase I and II studies of standardized extracts of black cohosh and red clover (*Trifolium pratense* L.) in menopausal women, tested these extracts for pesticide and herbicide residues as well as for heavy metals (23).

To assess the possibility of pesticide exposure to consumers of botanical dietary supplements, Huggett, *et al.* (24) used gas chromatography with electron capture detection to analyze a series of botanical dietary supplements for organochlorine pesticides. Between five and 12 samples each of valerian, St. John's wort, passion flower (*Passiflora incarnata* L.), and echinacea were obtained from commercial sources in the United States. The organochlorine pesticides aldrin, dieldrin, endrin, chlordane heptachlor, heptachlor epoxide, and DDT were detected in some samples. Many samples did not contain any detectable levels of organochlorine pesticides. The highest levels were 57.3 ng/g endrin in passion flower, 33.4 ng/g heptachlor epoxide in St. John's wort, 23.8 ng/g dieldrin in St. John's Wort, and 28.5 and 24.7 ng/g aldrin in St. John's wort and echinacea, respectively. Huggett, *et al.* (24) concluded that the presence of these pesticides at levels exceeding 20 ng/g in botanical dietary supplements indicate potential for hazard to human health depending upon the intake levels. It should be noted that the use of many of these pesticides are either banned or restricted in many countries including Canada, the United States and those of the European Union. Therefore, testing the plant material for pesticide residues prior to incorporation into dietary supplements or assaying the processed dietary supplement could control or eliminate this hazard, if highly contaminated materials were excluded from use or if the final product contained pesticide levels deemed safe for human consumption at the expected levels of intake.

Although plants cultivated using good agricultural practice or collected using good field practice should not be contaminated by pharmaceutical agents, subsequent processing in pharmaceutical facilities might inadvertently introduce pharmaceutical compounds into botanical dietary supplements. There is also the possibility of adulteration of dietary supplements by pharmaceuticals. One of the best documented examples of contamination of botanical dietary supplements by pharmaceutical agents was PC-SPES, which was a popular botanical combination used by men for the treatment of prostate cancer from 1996 until its withdrawal from the market in 2002.

The dietary supplement PC-SPES was a combination of seven botanicals and one fungus *Scutellaria baicalensis* Georgi, *Rabdosia rubescens* Hara, *Isatis indigotica* Fort, *Dendranthema morifolium* Tzvel., *Serenoa repens* Bartram (Small), *Panax pseudoginseng* Burk., *Glycyrrhiza uralensis* Fisch., and *Ganoderma lucidum* Karst. The name PC-SPES is derived from an abbreviation of "prostate cancer" combined with the Latin word spes meaning hope. Although PC-SPES showed anticancer activity in vitro (25) and in clinical trials (26), it was found to be contaminated by pharmaceutical compounds, some of which might exhibit anticancer activity. Using GC-MS, different lots of PC-SPES were tested and found to contain the potent synthetic estrogens diethylstilbestrol (25,27,28) and ethinyl estradiol (27), which can inhibit the growth and proliferation of androgen-sensitive prostate cancer cells. Additional analyses using GC-MS and LC-MS also identified warfarin and indomethacin (25,27) in some lots of PC-SPES. Subsequently, PC-SPES was removed from the market. This incident prompted calls for the application of good manufacturing practice and analytical quality assurance to prevent the sale of botanical dietary supplements contaminated or adulterated with pharmaceutical agents (25,28).

Standardization

After botanical material has been authenticated and the processed dietary supplement has been found to be free from hazardous contaminants, the next step to ensure a safe and reliable dietary supplement is standardization. The goal of standardization is to provide consumers with a product that contains consistent levels of active ingredients (chemical standardization) and predictable pharmacological and physiological effects (biological standardization). Reproducibility of the dietary supplement helps ensure safety by preventing accidental overdose due to lot to lot variation and by providing the consumer with predictable physiological and pharmacological efficacy.

If the active constituents of a botanical dietary supplement are known, then the product sold to consumers should be standardized to specific levels of these compounds. For example, Piersen *et al.* (23) standardized an extract of the aerial parts of red clover (*Trifolium pratense* L.) to 15% estrogenic and proestrogenic isoflavones consisting of deconjugated daidzein, genistein, formononetin, and biochanin A. Although not significantly estrogenic as administered, it was noted that formononetin and biochanin A are metabolized *in vivo* to form the much more estrogenic daidzein and genistein, respectively. This extract was then used in phase I and phase II clinical trials to establish the safety and efficacy of red clover in the prevention of symptoms such as hot flashes in menopausal women. As an example of chromatographic data that may be used as a basis for chemical standardization, Figure 2 shows a HPLC-UV chromatogram of the red clover extract used by Piersen *et al.* (23).

In some cases, the active constituents might not yet be known, and marker compounds that are unique to the particular species used to produce the dietary supplement may be used as surrogates during chemical standardization. An example is black cohosh which is used by women as a dietary supplement for the relief of menopausal symptoms. Although it was reported recently that black cohosh might relieve hot flashes in menopausal women by modulating serotonin receptors in the hypothalamus (29), the most active serotonergic compounds in this plant remain uncertain. Therefore, black cohosh is usually standardized to characteristic triterpene glycosides such as actein and 23-epi-26-deoxyactein even though these compounds have no serotonergic activity (30,31).

As a complement to chemical standardization, biological standardization should be used also to ensure the safety and reproducibility of botanical dietary supplements. Biological standardization should utilize quantitative assays that represent the desired efficacy of the dietary supplement. Since these assays should be economical, rapid and robust and reflect the underlying biological mechanisms of action, they are usually based on *in vitro* protocols, such as enzyme assays, receptor binding assays, gene expression assays, etc. Although expensive and low throughput, *in vivo* assays are sometimes carried out since they provide physiological relevance and incorporate contributions from bioavailability, metabolism and toxicity.

As examples of biological standardization, Piersen, *et al.*, (23) used both *in vitro* and *in vivo* bioassays to evaluate an extract of red clover prior to its use in clinical trials of safety and efficacy for the relief of menopausal symptoms in women. This extract had been standardized chemically to 15% isoflavone content after deconjugation of the isoflavones to their corresponding aglycons. The bioassays were selected to evaluate the estrogenicity of the extract, which was expected to be the primary mechanism of action in the relief of menopausal symptoms such as hot flashes. The *in vitro* bioassays included binding to the estrogen receptors- α and $-\beta$ in a cell-free system and cell based assays evaluating the induction of alkaline phosphatase in Ishikawa endometrial cells and up-regulation of the progesterone receptor and the trefoil peptide (TFF1/*pS2*) mRNAs in Ishikawa and S30 cells. The *in vivo* evaluation of the estrogenicity of the red clover extract was carried out using the Sprague-Dawley

ovariectomized rat model and included the morphological endpoints of uterine mass, cornification of vaginal cells, and mammary gland ductal branching (23,32).

When multiple botanicals are used in a dietary supplement, quality control can become an almost overwhelming challenge. Since complex mixtures of botanicals might have unique effects that cannot be achieved by just a few isolated chemical constituents, biological standardization of botanical dietary supplements might be preferred to chemical standardization in these cases. For mixtures of botanicals containing multiple constituents with related mechanisms of action, standardization using a single bioassay might be more cost effective than a battery of chemical assays for the individual active constituents. Furthermore, the quality control of dietary supplements containing mixtures of botanicals is complicated due to the batch-to-batch variation in the chemical composition of each botanicals used in the product. If the product can be standardized using bioassays instead of chemical assays, then this problem might become more manageable.

Conclusions

The consumer expects a botanical dietary supplement that is safe for consumption. The essential quality control and quality assurance procedures that the dietary supplement industry should follow to ensure the safety of botanical dietary supplements have been described in detail above and are summarized in Figure 3. Additional information has been described by Fong *et al.* (6) and Shiltler *et al.* (32). These procedures include acquiring the botanicals from growers or collectors who use good agriculture and collection practices. To be certain that the correct species has been acquired, the material should be authenticated using macroscopic and microscopic botanical examination. Alternative authentication assays include genetic identification using PCR techniques, immunoassays to identify species specific proteins, or chemical analysis for unique marker compounds. After processing, the botanical dietary supplement should be assayed for hazardous contaminants such as pesticides, herbicides, heavy metals, mycotoxins, and microbes. In addition, pharmaceutical contamination or adulteration should be ruled out by chromatographic assays designed to detect drugs that might have been added either inadvertently or deliberately during processing. Finally, the botanical dietary supplement should be standardized both chemically, based on the concentration of active compounds (or marker compounds if active constituents are unknown), and biologically, based on bioassays for known or desired pharmacological and physiological effects. These final standardization steps will assure the consumer of a reproducible product. In addition to these basic steps to ensure the safety of botanical dietary supplements, more advanced toxicity tests that are beyond the scope of this review should be carried out that include preclinical and clinical studies as described by Fong, *et al.* (6). Although these procedures will probably be implemented over a long period of time, they will be essential to help ensure the safety of botanical dietary supplements.

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Figure 1.

Positive ion electrospray LC-MS-MS analyses of extracts of *Panax ginseng* C.A. Meyer and *Panax quinquefolius* L. showing that these species may be distinguished by the ratio of ginsenoside Rf and 24(R)-pseudoginsenoside F₁₁. Reversed phase HPLC separations were carried out using a C₁₈ column, and multiple reaction monitoring with collision-induced dissociation were used during tandem mass spectrometry as described by Li, *et al.* (15).

Figure 2.

Reverse phase HPLC chromatogram obtained using UV absorbance detection at 254 nm of an ethanolic extract of the aerial parts of red clover (*Trifolium pratense* L.). The extract was chemically standardized to the estrogenic isoflavones daidzein and genistein and the pro-estrogens formononetin and biochanin A and used in clinical studies of the safety and efficacy of red clover dietary supplements for the relief of menopausal symptoms in women. For more details, see Piersen, *et al.* (22).

Figure 3. Quality assurance and quality control of botanical dietary supplements depend upon an array of assays and procedures that must be followed under proper guidelines to assure the safety of the consumer.