Quality assessment of medicinal herbs and their extracts: Criteria and prerequisites for consistent safety and efficacy of herbal medicines

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Abstract
Ingredients of commercial herbal medicines are assessed for quality primarily to ensure their safety. However, as complex mixtures of different groups of plant secondary metabolites, retention of overall phytochemical consistency of herbal medicines is pivotal to their efficacy. Authenticity and homogeneity of the herbs and strict regimes of physical processing and extract manufacturing are critical factors to maintain phytochemical consistency in commercial products. To ensure both safety and efficacy of herbal medicines, implementation of and adherence to good agricultural and collection practice (GACP), good plant authentication and identification practice (GPAIP), good manufacturing practice (GMP) before and during the manufacturing process, and good laboratory practice (GLP) in analysis are necessary. Establishment and application of harmonized multilaboratory-validated analytical methods and transparency in the supply (value) chain through vendor audits are additional requirements in quality assurance. In this article, we outline steps of a comprehensive quality assurance paradigm aimed at achieving and maintaining safety, consistent phytochemical composition, and clinical efficacy of ingredients of herbal medicines.

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1. Introduction
The ingredients used in commercially marketed traditional and modern herbal medicines, dietary supplements, functional foods, and “super” foods are today mostly assessed for quality to ensure their safety. However, there is a need for a quality insurance paradigm for botanicals that will address both safety and efficacy. As Bauer and Tittel pointed out almost 20 years ago, “since phytotherapy is a regular part of medical treatment, its efficacy and safety should be obligatory, and the need of pharmacological, toxicological, and clinical trials is obvious” [1]. Efficacy and safety of herbal medicine finished products are directly dependent on the quality and chemistry of medicinal herb raw materials. However, the phytochemical profiles of the medicinal herb raw materials are qualitatively and quantitatively variable and to define the quality of medicinal herb raw materials as well as the terminology used vary between countries. Therefore, it is important to define quality criteria that are intricately linked to constancy of phytochemical profiles. Strict adherence to the harmonized quality assessments will enable consistent pharmacological activity and facilitate meaningful comparison and meta-analysis of clinical data.

Globally, a number of terms, definitions, and quality paradigms are used based on the intended use of herbal products and the types of health claims associated with the final product (Table 1). Herbal products are marketed as complementary/alternative/nonconventional medicines (CAM), which are often based on traditional medicines such as traditional Chinese medicines, Indian Ayurvedic and Siddha medicines, and Japanese Kampo medicines, for example, and as nutraceuticals, dietary supplements, and more recently, as functional foods. However, quality requirements and health claims for herbal ingredients differ between these categories. This raises the following question: should there be different quality criteria for herbal ingredients when used in different product categories? Or should all herbal ingredients be subjected to the same quality criteria irrespective of the health claims?

1.1. Comparison of national quality requirements and levels of health claims
Levels of allowed health claims and, in turn, the quality requirements for botanicals in different categories are delimited by national regulatory policies. Such legal diversity, especially among countries with long histories of traditional use of botanicals, makes the establishment of internationally harmonized quality guidelines quite difficult.
In Germany, herbal medicinal products including traditionally used herbal medicines are integrated into primary health care. A simplified registration process has been established [2] in which herbal medicinal products have to be evaluated for safety and efficacy and produced in an environment strictly adhering to good manufacturing practice (GMP). As a part of “quality requirement”, a committee on herbal medicines was established (Commission E), which produced monographs on specific herbs that form the basis for outlining their quality, efficacy, and safety. To date, ~380 Commission E monographs covering 360 plant species have been published [3]. This simplifies the registration procedures of traditional herbal medicines across the European Union.

In Australia, botanicals are used mostly in listed “complementary medicines”, which are considered to carry only a “low risk” of adverse effects. The Australian regulatory guidelines for complementary medicines (ARCGM) [4] provide compositional guideline requirements for herbal medicinal products as well as herbal raw materials and preparations. They also provide guidance on test procedures and criteria for acceptance or rejection to assure the quality of herbal substances. In order to ensure quality, application of GMP process controls and validations have to be applied throughout the development process. The ARCGM herbal ingredients — quality guidelines are essentially an adaptation of the European Medicines Agency (EMEA — CPMP/QWP/2820/00) document “Test procedures and acceptance criteria for herbal drugs, herbal drug preparations, and herbal medicinal products”.

In China and India, traditional herbal medicines are fully integrated into mainstream health care and, hence, treated as regular medicines. Herbal medicines in China are classified either as functional foods or as drugs. Quality requirements and efficacy claims differ between them. However, herbal medicines are covered under Chinese drug laws. In order to develop proper drug standards for herbal medicines, the Pharmacopoeia of the People’s Republic of China (2010 Edition) provides over 2136 monographs [5].

A comprehensive draft guidance document, “Quality of National Health Products Guide”, is provided by the Natural Health Products Directorate, Canada. This document outlines quality requirements for medicinal herb preparations in Canada [6] and provides details on the required characterization, identification, and quantification of standards. Health Canada also provides a number of Natural Health Products Ingredients Database (NHPID) monographs that include “single ingredient monographs” for medicinal herbs [http://webprod.hc-sc.gc.ca/nhpid-bdipsm/monosReq.do?lang=eng].

In the United States, the Dietary Supplement Health and Education Act [7] permits claims of functional specific health benefits for dietary supplements. However, it does not allow claims on specific disease prevention or cure [8]. The manufacturer of the dietary supplement is responsible to ensure ingredient quality and safety. In 2007, the Food and Drug Administration (FDA) provided GMP guidelines for dietary supplement manufacturing. However, raw material (ingredient) manufacturing facilities are exempt from the new FDA GMP guidelines and the burden of quality assurance of ingredients rests with the dietary supplement manufacturer. The FDA does not stipulate accurate chemical analyses of ingredients as a basis for identification and quantitation. This means that individual dietary supplement manufacturers will determine and apply their own definition of quality specification of ingredients. Accordingly, the exact specifications will differ among manufacturers and “can (with very few exceptions) be as loose or as tight as determined by each manufacturer” [9].

The Therapeutic Goods Administration (TGA) in Australia has provided compositional guidelines for herbal substances for use in “listed medicines”. This is a good starting point document for herbal ingredients’ quality in traditional medicines, dietary supplements, nutraceuticals, and functional foods. A critical requirement is the ingredient specification, which includes identity tests, marker quantity, purity, potency, and the required acceptance criteria for each of these test parameters [6]. Almost all national guideline documents place a central emphasis on the utility of their major national pharmacopeias such as the United States Pharmacopeia (USP; http://www.usp.org), British Pharmacopoeia (BP; http://www.pharmacopoeia.co.uk), European Pharmacopoeia (EP; https://www.edqm.eu/en/european-pharmacopoeia-8th-edition-1563.html), Chinese Pharmacopoeia (CP) [5], Japanese Pharmacopoeia (JP; http://www.pmda.go.jp/english/pharmacopoeia/index.html), and American Herbal Pharmacopoeia (AHP; http://www-herbal-ahp.org/index.html) and the individual herb monograph references in them to refer to in the herbal ingredient specifications and their acceptance criteria. However, it should be noted that the European Pharmacopoeia provides only herb monographs, while others include as well monographs on herbal extracts and other herbal preparations. The number of herb extract/preparation monographs in major pharmacopoeias is relatively small. Availability and utilization of multiple manufacturing processes and different extract specifications globally make it difficult to provide harmonized monographs for herbs and their preparations.

2. The primary steps in quality assurance

2.1. Classical systematics in species authentication and the utility of good agricultural and collection practice (GACP)

In Australia, Canada, Europe, and the USA, ensuring the quality of medicinal herb ingredients (raw materials) and complying with the respective national regulatory framework lie with the product license holder (sponsor of the final medicinal herbal formulation). The sponsor of the herbal medicine, in turn, has to place specific systems and requirements for approval of ingredients (raw materials) through the supply (value) chain back to the ingredient source and manufacture to assure quality. Many but not all international firms tend to adopt vendor audit programs to qualify herb source and ingredient manufacture by stringent GMP protocols.

Quality assurance of medicinal herb ingredients throughout the supply chain begins with the sourcing of authentic herbs. Authenticating the starting (whole herb) material to the species level is a fundamental requirement to establish the purity of ingredients derived from it across...
the supply chain. At present, medicinal herbs are sourced either from organized cultivation or by wildcrafting. However, trading of dried and sorted medicinal herbs in the market place with no traceability to their origins is quite common and makes authentication often difficult or outright impossible.

The medicinal herb industry worldwide, especially herbal ingredient manufacturers, relies on herb identification as described in pharmacopeial herbal monographs. Pharmacopeial identification descriptors begin with dried, unprocessed "whole/fragmented/cut/chopped plants or plant part". The ingredient identity is based on macroscopic, microscopic, and organoleptic analysis of sorted and dried plant parts. On the other hand, whole plant specimens (including the flowering and fruiting parts) provide information on crucial character sets including leaf shape, size and type (compound or simple), phyllotaxy, floral characters, and arrangement in inflorescence which may be irretrievably lost when the material is fragmented or sorted. Hence, these monographs, at best, provide an identification tool, which is relevant only when the whole plant material is authenticated prior to sorting at the source.

In classical systematics, plant species are delineated, described, and authenticated based on multiple vegetative, floral, and fruit (phenotypic) character sets. In some diffuse taxa, additional morphometric characters are used to authenticate species. In order to arrive at proper authentication, multiple whole herb samples (with flowers and fruits) from the bulk harvest and retained voucher specimens (in the form of herbarium specimens) should be examined and compared with species descriptions by taxonomic specialists. Such source authentication of whole herbs should be the basis of any claim in regard to the purity of ingredients derived from them. Yet another way to ensure source authentication of the bulk herb harvests is by good agricultural practice (GAP) to cultivation, which is rather limited. Wildcrafting of medicinal plants is a common practice; however, stringent adherence to good collection practice (GCP) is most often not applied.

Good agricultural and collection practice (http://www.ahpa.org/portals/0/pdfs/06_1208_ahpa-ahp_gacp.pdf) [10–12] for medicinal plants partly addresses issues related to plant authentication. For instance, GAP recommends that the identity of botanicals be authenticated at the species level and that of their propagules (seeds or other planting stocks such as rhizome and vegetative cuttings) at the subspecific level (variety, cultivar, and hybrids) to ensure material homogeneity of the bulk harvests (for details, refer to Table 2). Wildcrafting of medicinal herbs, especially from forested areas, is often a random exercise, and organized collection of medicinal herb species under supervision by a systematic botanist is often limited. In the case of wildcrafting of medicinal herbs, good collection practice (GCP) describes a set of guidelines, which include permits and permissions for collection, site selection, safety of collection equipment, plant authentication (to species), and harvest times based on phenology and sustainability. The main concern with wildcrafting is on the harvester qualification and training in taxonomy identification that is decisive in ensuring the purity of the bulk harvests, as authentication protocols are placed only on random herb samples.

Good agricultural practice ensures herbal material homogeneity based on phenotypic characteristics. The main variables are climatic, soil-related (edaphic), and genetic influences on phenotypes. With GCP, the variables include, in addition, subspecific heterogeneity and lack of bulk authenticity. These variables influence the phytochemistry of the herbs and, consequently, that of their raw extracts.

Ambiguities in defining a species as well as changes in taxonomic concepts quite often lead to reorganization of taxa. For example, correct species identification of medicinal genera such as Artemisia, Astragalus, Rubus, Crataegus, Mentha, Organum, and Salix poses difficulties even to trained systematic botanists [13,14]. Taxonomists add sometimes to the confusion by raising a subspecies to the species level or merging of a number of species to just a single one. A case in point is Viburnum opulus, a subgeneric complex, under which the taxa are variously treated as species or subspecies by different taxonomists.

Table 2

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Good authentication and identification practice (GAP) for medicinal plants is recommended as an important step for medicinal species authentication. Establishing standard operating procedures (SOPs) for collection and preparation of medicinal plant voucher herbarium specimens is the first step in GAP. Whether cultivated or wildcrafted, the voucher should correctly represent the bulk biomass as it serves to document the bulk biomass species authenticity [15]. Voucher specimens should be harvested or collected from the same population or location at similar times and should bear a unique identifying number. For each harvest/collection, date, names of collectors/harvesters, geographical coordinates, habitat, population size, fresh plant attributes (color, odor, habit, size of plants), and cultivar name (if cultivated) should be recorded in field notes in order to prepare proper labels for the voucher herbarium sheets. Comprehensive information on SOPs for preparation of voucher specimens for herbarium deposits has been discussed in the literature [15,16]. Retention of vouchers for each batch of bulk biomass enables verifiable identification and allows rechecks downstream in the supply chain. It also enables the study of batch consistency and reproducibility of derived products by phytochemical profiling. Voucher deposits should be compared with a reference voucher in local herbaria and species-defining character sets with published species descriptions to confirm authenticity. An expert taxonomist is required to certify this step and provide a certificate of botanical authenticity of the specimen.

2.2. Genomic profiling and DNA barcoding in species authentication

Genomic profiling and DNA barcoding are complementary techniques to classical systematic approaches to plant authentication, which can be used for the unequivocal identification of plant species [17]. Herbert et al. coined the term ‘DNA barcode’ in 2003 [18]. A DNA barcode is a short gene sequence from a specific region of the genome. The Plant Working Group of CBOL (Consortium for the Barcode of Life) was promoted to develop DNA barcoding in plants in 2004. Based on the extensive literature, it was realized that a single locus barcode might not be sufficient for plant species authentication. The CBOL recommended two-locus combination for authentication. They are chloroplast genes mature K (matK) and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) [19]. The need to include a third locus (internal transcribed spacer or nuclear ITS) to achieve maximum identification rates is currently being debated [20–22]. Deoxyribonucleic acid barcodes should exhibit sufficient variability for
species identification, and the system should be amenable to easy referencing. Recovery of the DNA barcode from herbarium samples or processed samples (as in medicinal plant powders) should be made possible, and absolute barcode sequences should not overlap between species. However, DNA barcoding is a work in progress. There are a number of challenges before DNA barcoding can be used as a routine quality paradigm for plant species authentication [19].

In addition, there are challenges in the practical application of DNA barcoding methods for medicinal plant authentication (Fig. 1). Sourcing appropriate fresh plant parts from authenticated whole plants is the most critical step for proper DNA extraction. Existing barcoding systems are not standardized or standardizable to a wide range of plant species [23] and what method to choose has to be decided on a case-by-case basis. A reference database that contains verified sequences of correctly identified plants and a query system that can properly match an unknown to the correct species is required [24]. Such a dedicated database system does not exist, and an incomplete reference dataset will have lesser probability of proper matching [19].

2.3. Macroscopic and microscopic characterization of sorted plant materials

Two additional datasets for medicinal plant authentication are macroscopic and microscopic character sets of sorted plant parts and their phytochemical (metabolite) profiles. Most pharmacopeial monographs, in fact, begin with the identification of the species based on the morphological and anatomical characters of dried and sorted plant parts such as roots (e.g., Panax ginseng and Hydrastis canadensis), leaves (e.g., Ginkgo biloba and Hamamelis virginiana), berries/fruits (e.g., Crataegus monogyna and Silybum marianum), bark (e.g., Rhamnus frangula and Salix alba), flowers (e.g., Marrubium vulgare and Lavandula angustifolia), seeds and husks (e.g., Plantago ovata), or derived products such as gum/resins (e.g., Boswellia serrata and Commiphora mukul). The monographs also provide identification markers for the microscopic examination of plant parts, including protocols for preparation of sections (treating with chloral hydrate [25] as a clearing agent and staining, if necessary). Cell types, cell inclusions such as calcium oxalate crystals and crystal structures, vessel types, pittings and thickenings, and tracheids are some of the features studied using the microscope to ascertain the identity of the material. It has to be borne in mind that species-specific characters in sorted plant parts are rare, making authentication by both macroscopic and microscopic characters at the postprocessing stage difficult. Identification methods and practices for sorted plant parts, hence, should be viewed rather as verification of a previously authenticated (by classical taxonomy and DNA methods) whole plant from which the examined part is derived.

2.4. Phytochemical profiling of plant parts as a tool for identification and characterization

Phytochemical profiling (metabolite fingerprinting) is increasingly used for species- and plant part-specific identification [26]. Phytochemical profiling primarily employs high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography in addition to gas chromatography (GC) and capillary electrophoresis (CE). Ultraviolet (UV) and visible (Vis) light-based photodiode array (PDA) detectors and mass spectrometry (MS)-based detectors are commonly used. Among these techniques, thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC), qualitative and semiquantitative techniques, are extensively used in the medicinal herb industry for the generation of chemical fingerprints. The technique utilizes UV absorption characteristics at two different UV wavelengths (254 nm and 366 nm) and derivatization reagents to
provide chromatograms of separated constituents (Fig. 2). Phytochemical profiling of a large number of medicinal herb species (and their plant parts) has been reported in the literature [19,27–29] including images for comparison. Methods and chemoprofiles have also been provided by CAMAG (http://www.camag.com/en/tlc_hptlc/camag_laboratory/methods.cfm, a manufacturer of TLC/HPTLC equipment) and by the HPTLC Association (http://www.hptlc-association.org/methods.cfm) for a number of medicinal herbs, which are freely available on the Internet.

Phytochemical profiling by HPLC for species (and plant part) identification is gaining importance. Single UV wavelength detection is mostly used. The generated chemoprofiles provide a multiconstituent profile pattern that provides a basis for discrimination among samples facilitating correct identification (Fig. 3). Species- and plant part-based HPLC chemoprofile libraries are not as extensively available in the literature as in the case of HPTLC fingerprinting. Wagner et al. [28] have attempted to provide HPLC chromatograms with specific marker compounds for some traditional Chinese medicinal herbs. Chemoprofiling for identification is specific to the plant part of a species because of differences in spatial distribution patterns of phytochemistry and is also governed by temporal factors (age of the plant). Equally important is the type of solvent used for sample preparation for analysis (polarity based). All these factors determine the final profile. Hence, the method of analysis should be properly validated prior to generating the profile for use in identification.

Imprecise terms such as herb, aerial parts, herb flowering top, herb flowering and fruit, leaf and stem, secondary roots, and rhizome/stolon are often used in the industry. A clear statement on the plant part(s) used within the strict botanical definition is a must prior to the application of phytochemical profiling for identification. For example, ‘herb’ is construed as all aerial parts, without giving details of flowering and/or fruits. ‘Herb flowering top’ and ‘herb flowering fruit’, stems, and leaves are used without the compositional (w/w) ratios of biomass. A clear (botanical) definition of the specific plant part(s) used, with compositional ratios of different plant parts, if any, must be provided in the ingredient specification for the utility of chemoprofiles for identification.

2.5. Guidelines for good plant authentication and identification practice (GPAIP)

Agriculture and Agri-Food Canada has provided an excellent guideline document on good practices for plant identification for the herbal industry [30]. As an extension to this, we recommend following GPAIP for the medicinal herb industry because the medicinal herb ingredients undergo change hands frequently during various stages of processing and their journey across the supply (value) chain (refer to Table 3).

Postphysical processing (drying, sorting, and milling) and prior to extract manufacture, the medicinal plant material can be identified by microscopic, organoleptic, and phytochemical characteristics. This should be sequel to source herb authentication by GPAIP.

2.6. Assurance of the ‘purity’ of botanical raw materials: impurity profiling

Ensuring authentic medicinal herb starting materials free of impurities is the next major step in achieving quality end products. Impurities include heavy metal contamination, pesticide residues, and aflatoxins/mycotoxins.

The source of heavy metal contaminations in medicinal plant species is the cultivation or collection from contaminated soils. Over 500 plant species (~0.2% of all angiosperms) are known to hyperaccumulate heavy metals [31,32], and in some species, heavy metal concentrations in aerial parts exceed critical toxicity levels. Heavy metal hyperaccumulation is associated with metal hypertolerance in some plant species that tend to grow well in contaminated soils [31,33]. Ni, Cd, Se, As, Mn, Co, Cu, Pb, Sb, Ti, and Hg are the metals and metalloid species that are hyperaccumulated. Examples of medicinal plant species accumulating heavy metals include members of Brassicaceae [31], Matricaria recutita, Hypericum perforatum [34], Carthamus tinctorius, and Trifolium pratense [35], to mention a few. The herbal medicine industry worldwide tests for heavy metals (Pb, Hg, As, and Cd), and the preferred techniques are atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and neutron activation analysis (NAA). The limits for the heavy metals and metalloids in medicinal herb starting materials and preparations are provided based on the provisional tolerable intake (PTI) values. Individual countries specify either quantitative assays or limit tests, and details of limits of PTI values are provided by the WHO, AHPA, and individual herbal monographs of British and US pharmacopoeias [36,37]. Good agricultural practice recommends soil analysis (metal and metalloid content) as a first step in the choice of land for cultivation that eliminates heavy metal contamination in medicinal herbs.

Pesticide residues in medicinal herbs arise from crop protection practices in cultivation. ‘Pesticides’ include fungicides, insecticides, and weedicides. Types of pesticides used vary with individual countries’ regulations and the target pests (fungal/microbial pathogens, insect pests,
and weeds) encountered in the agroecosystem. An array of different classes of pesticides such as organochlorines, organophosphorus, carbamates, benzimidazoles, dithiocarbamates, and amino acid herbicides are used in plant protection. Persistence of different pesticides in the environment differs. It is advisable to record the use of specific pesticides and their concentrations and time of application for each cultivation season (to understand the length of exposure). Such information is required to apply appropriate analytical techniques to accurately quantify them in medicinal herb starting materials. When testing for a medicinal plant material of unknown cultivation practice, it is desirable to test for all the broad groups of pesticides. A number of methods are available to test for organically bound chlorine, phosphorus, arsenic, lead, and carbon disulfide to predict and assay broad groups. Pesticide residue assessment in medicinal herbs of unknown origin is a major cost driver in quality assurance downstream in the supply chain. Good agricultural practice documents the pesticides used, their concentration, and the time of application, facilitating application of appropriate methods for quantitative assessment of pesticide residues. Most pharmacopeias recommend appropriate methods in the individual herbal monographs.

Aflatoxins and mycotoxins are fungal secondary metabolites produced by species of *Aspergillus, Penicillium, Fusarium,* and *Alternaria* [38]. Among the 400 known mycotoxins, aflatoxins, ochratoxin A, fumonisins, zearalenone, and deoxynivalenol are the most important ones. Mycotoxins are carcinogenic, neurotoxic, teratogenic, and immunotoxic. A number of medicinal plant species including chamomile, garlic, ginger, milk thistle, kava kava, Indian senna, turmeric, ginseng, and licorice roots tend to harbor mycotoxin-producing fungi during harvesting, handling, and storage because of poor agricultural and harvesting practices [39,40]. Most pharmacopeias provide safe limits of aflatoxins and ochratoxin A in individual medicinal herb monographs. A number of validated methods of mycotoxin analysis in the medicinal plant matrix are available in the literature [40–42]. It is essential to prevent fungal infection and establish that the herbal material is free of mycotoxin contamination by appropriate postharvest and storage practices.

### 3. GMP in extract preparation

#### 3.1. Standardization and phytochemical profiling as quality measures

Other than in traditional herbal formulations (TCM, Ayurveda, Siddha), powdered medicinal herbs have limited use in modern herbal medicines. The vast majority of herbal ingredients in the global market place are soft or dry extracts. The only identification method available at the extract ingredient stage in the supply chain is chemoprofiling. The extract chemoprofile as ID will depend on 1) the time of harvest (to control temporal variations), 2) the plant part(s) and their compositional ratios (to control spatial variations), 3) solvents used in extract manufacture, and 4) the manufacturing method employed.

Medicinal plants elaborate a large number of phytochemicals of different polarities. The solvent(s) used in the extract manufacturing process results, on the one hand, in the enrichment of selected constituents (based on polarity) and, on the other hand, in the elimination of a number of other compounds. Such selective enrichment facilitates the reduction of phytochemical complexity and variability of extract ingredients. Medicinal herb extract preparations are either targeted (standardized) or nontargeted (nonstandardized). In nonstandardized extracts prepared by a consistent extraction process, chemoprofile variations are directly linked only to the variation of the composition of the starting material. On the other hand, in standardized extracts, chemoprofiles are influenced by both the variable phytochemical composition of the starting material as well as the choice of manufacturing process that is designed to enrich the extract in one or several specific constituents considered as markers and/or bioactives. This is primarily due to the varying ‘marker/active’ concentrations in herb starting materials sourced from different regions and seasons that may require different levels of enrichment to achieve the required concentration. To account for such ‘marker/active’ quantitative variation, allowance in the native extract ratio is given in range (for example, instead of an exact 100:1 ratio, a range such as 95–105:1).

There are no harmonized guidelines on the choice of marker(s)/active(s) for standardization of herbal extracts and preparations. In

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Table 3

Good authentication and identification practice (GARP) for medicinal herbs — what is required.

- A validated guidance document for ‘good plant authentication practice’ for medicinal herbs in consultation with plant taxonomy experts (GIM — general identification method for a given taxon)
- Preparation and retention of herbarium vouchers (standard operating procedures; SOPs)
- Certificate of botanical authenticity by a specialist taxonomist
- Herbarium reference number
- Information on subspecífic taxon (variety/cultivar)
- DNA barcoding (genomic profiling) — GAM (general analytical method)
- Document to link to the bulk (harvested/collected) medicinal herb batch
- GMP documentation on the medicinal herb batch
- SOPs and GTM (general technical methods) for the batch process — drying, sorting, and cutting of whole plant and plant part to specified size for further processing
- A validated guidance document for ‘good identification practice’ for sorted/powdered plant materials
- Macroscopic and microscopic identification methods (general analytical methods) for a given plant part of a specific taxon — pharmacopeial identification methods are a good starting point
- Chemoprofiling as an identification tool for the plant part of a specific plant taxon — utility of pattern recognition (GTM — HPTLC/HPLC/CC/CE)
- Batch documentation for organized cultivation/GAP/wildcrafted herbs
most cases, they are major components in an active extract or preparation. Examples include gingko flavone glycosides in G. biloba extracts, bacosides in Bacopa monnieri, hydroxyanthrancene derivatives in Frangula purshiana (Cascara) and in Senna alexandrina (senna), flavonolignans in S. marianum (milk thistle), and isoflavones in Glycine max (soy), to cite a few. Active extract ingredients are also standardized to a single active constituent, which may partly contribute to the total efficacy, such as andrographolide in Andrographis paniculata extracts, protodioscin in Tribulus terrestris, trans-resveratrol in Fallopia japonica, glycyrrhizin in Glycyrrhiza glabra (liquorice), icarin in Epimedium sagittatum, and forskolin in Coleus forskohlii. Examples of multiple ‘actives’ in an active extract are oligomeric proanthocyanidins in tea and grape seeds, curcuminoids (curcumin and its demethoxy and bisdesmethoxy derivatives) in Curcuma longa, eight ginsenosides in P. ginseng, and withanolides in Withania somnifera.

In addition to being quality indicators in standardized extracts, a number of these markers, either alone or in combination with similar constituents, can serve as identification tools. This is referred to as the "compound-oriented approach" to herbal extract identification [42]. Marker compounds are further subclassified into "species-specific markers" (e.g., silybin and isosilybin in S. marianum, andrographolide in A. paniculata, and rosavins in Rhodiola rosea) and "nonspecific phytochemical markers" (e.g., salidroside in Rhodiola species, salicin in Salix species, and harpagoside in Harpagophyllum that are also reported in several other plant species). The presence of the marker(s) individually or in combination provides confirmation on the ‘inclusion’ of a specific medicinal herb extract; however, it does not guarantee extract purity. Profiling multiple phytochemical markers in herbal extracts as a measure of quality is gaining ground. For instance, pattern recognition of extract HPLC/CC chromatogram profiles with either clearly identified marker profiles or profiles of all the detectable constituents is an excellent tool in quality assurance of medicinal herb preparations. For example, the flavonoid markers (epimedin A, B, and C and icarin) are used to assure the quality and identity of Herba epimedii, a Chinese remedy to treat impotence, bronchitis, and coronary diseases [44], with interchangeable Epimedium sagittatum, Epimedium brevicornum, Epimedium pubescens, Epimedium wushanense, and Epimedium koreanum [45]. Similarly, ginsenoside HPLC profiles of medicinal ginseng (Panax ginseng, Panax quinquefolia, and Panax notoginseng) are used to assure their individual identity and quality based on marker assays and profile patterns. Pattern recognition among HPLC chromatographic profiles that target specific chemistries has been successfully applied to delineate medicinal species of Taxus wherein thirty-three taxane peaks of interest were profiled [46].

Chemometrics, the application of mathematical methods in the analysis of chemical data, is used extensively in pattern recognition [47]. By a ‘preprocess’, multiple chromatograms are aligned by retention time corrections. The processed data are then subjected to principal component analysis (PCA) to reduce large numbers of correlated variables (e.g., peak height measurement) to a smaller number of uncorrelated variables (principal components). The data are then plotted in relation to 2 or 3 principal components on a 2- or 3-dimensional plot, respectively. A score plot allows a quick identification of similarity. Hierarchical cluster analysis (HCA) in conjunction with PCA provides classification of sample groups based on the Euclidean distance [48]. A ‘loading plot’ is useful to determine the specific compounds (peaks) responsible for any observed variation among samples.

Pattern-oriented multicomponent (or holistic) chromatographic profiling and application of chemometrics provide information on extract identification (to the species and plant part). Application of chemometrics to Equisetum arvense extract profiles from different parts of the world showed distinct differences in profile patterns, establishing subspecific differences due to phytogeographical influences [49]. It also amply demonstrated the need to source the same subspecific chemotype to have profile consistency to ensure efficacy consistency in species that show larger subspecific variations. Principal component analysis also provides clues to identify adulteration (if unknown impurities are detected). The method can also be successfully employed to generate information on ‘multicomponent’ extract stability in storage, hitherto not available in the industry.

3.2. Analytical method harmonization throughout the supply chain

Lack of harmonization in quality assessment methodologies is a major impediment in quality assurance. Development of reliable and validated analytical methods for botanical ingredient identity, purity, and strength has not kept pace with the rapidly expanding number of botanical ingredients in the market place. Lack of validated analytical methodologies in tune with complex ingredient chemistry has stymied the much needed research in efficacy correlations. Governments and conscientious self-regulating industry leaders in botanicals have initiated efforts to set quality standards by developing and adopting validated analytical methods for medicinal herb ingredients [50]. The International Conference on Harmonisation (ICH) provides ground rules for validation of analytical methods (http://www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures-text-and-methodology.html). A number of regulatory bodies including the Australian TGA (https://www.tga.gov.au/starting-material-analytical-procedure-validation-complementary-medicines-0) recommend the use of pharmacopeial methods or published methods already subjected to validation as per ICH and/or NSF international guidelines.

Analytical method validation ensures the method suitability and ruggedness as a quality measure across multiple laboratories. In turn, a product is verified for assay of desired analyte(s) to the specification and to identify contaminants. The AOAC (Association of Official Analytical Chemists) International in the USA, for example, has taken up single-laboratory validation for a number of botanical ingredients (in dietary supplements) that are subsequently adopted by multiple laboratories for cross-verification and further validations. Guidelines for validation of qualitative (semiquantitative) chromatographic methods such as HPTLC for fingerprint analysis and identity and impurities profiling are also available in the literature [27]. A number of international botanical ingredient suppliers have developed analytical methodologies, single-laboratory validations, and collaborative multilaboratory validations following ICH guidelines for a number of botanical ingredients and have published them in peer-reviewed journals. Collaborative efforts in validation also find place in a number of pharmacopeial monographs for botanical preparations. Guidelines for validated method transfer are also available and facilitate the adoption of method harmonization across the supply chain. However, the widespread industry practice of using varying ingredient strengths (marker and extract ratios) sourced from single species (and plant parts) complicates the adoption of a single validated analytical method in a quality control laboratory. This is especially true of contract manufacturers of polyherbal formulations catering to multiple sponsors, each requiring different strengths of botanicals in their products.

3.3. Transparency in the supply chain and comprehensive quality assurance

Another major challenge in quality assurance of botanicals from farms to medicines is to provide transparency throughout the supply chain because of international diversity in national regulatory policies. This is addressed by qualifying vendors (starting herb supply chain, manufacturer of raw material, and finished product manufacturer) by a qualification process, namely the vendor audits. Supply chain transparency, when properly documented, provides stringent quality assurance at each link of the supply chain that is verifiable by repeated vendor audits. Regulators in the European Union and Australia have begun to emphasize vendor auditing of sourcing and production facilities in different countries as a requirement of GMP. Good agricultural practice, application of codes of GMP in manufacture, GLP, harmonization of method protocols, and supply chain transparency will provide
the much needed quality assurance of botanical ingredients. Homogeneous starting materials, validation of manufacturing protocols, and application of harmonized analytical methods throughout the supply chain will ensure optimal levels of phytochemical consistency that can be monitored by application of chemometrics. Such a paradigm will ensure authentic, phytochemically comparable, and adulteration- and impurity-free herbal ingredients from which evidence for chemistry–ef
cfficacy correlations in herbal medicines can be scientifically collated. We have included a figurative value (supply) chain–herbal ingredient quality assurance paradigm as envisaged (Fig. 4) for use in complementary medicines, nutraceuticals, dietary supplements, and functional foods.

4. Conclusions

The present quality assurance models for medicinal plant preparations for use in herbal medicines, dietary supplements, functional foods, and nutraceuticals vary depending on health claims and diverse national regulatory policies. What is required is international harmonization of quality regulation and transparency throughout the supply (value) chain of botanical ingredients. The present paradigm of safety as the (only) basis for quality assurance has to be amended to include evidence-based, phytochemistry-related efficacy. Fundamental to this more comprehensive quality paradigm is the assurance of authenticity and homogeneity of the medicinal herbs, validated extract manufacturing protocols, harmonized and validated test methodologies, and transparency at all levels of the supply (value) chain. We advocate adoption of a comprehensive paradigm that incorporates GPAIP for species and subspecific authenticity, GAP for homogeneity of medicinal herbs, GMP for extract manufacturing, GLP for harmonized test methodologies, and vendor audits to establish supply chain transparency. We have provided specific quality requirements at each point throughout the supply chain to establish authenticity, homogeneity, and purity to achieve consistent quality of medicinal herb preparations. We have outlined the importance and utility of the implementation of classical systematics, genomic authentication, macro- and microscopic characters of sorted plant materials, marker (actives) identity and assay, chemoprofiling and pattern recognition, multicomponent stability protocols, impurity profiling, and analytical method validation/harmonization in a comprehensive quality assurance paradigm.

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Conflict of interest

The authors declare that they have no conflict of interests.

References


Fig. 4. The value chain and envisaged herbal ingredient quality paradigms. GACP: good agricultural and collection practice, GPAIP: good plant authentication and identification practice, and GMP: code of good manufacturing practice. *Aflatoxins/mycotoxins, heavy metals, pesticides, and adulterants.