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Integrated analytical assets aid botanical authenticity and adulteration management

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Abstract

This article reviews and develops a perspective for the meaning of authenticity in the context of quality assessment of botanical materials and the challenges associated with discerning adulterations vs. contaminations vs. impurities. Authentic botanicals are by definition non-adulterated, a mutually exclusive relationship that is confirmed through the application of a multilayered set of analytical methods designed to validate the (chemo) taxonomic identity of a botanical and certify that it is devoid of any adulteration. In practice, the ever-increasing sophistication in the process of intentional adulteration, as well as the growing number of botanicals entering the market, altogether necessitate a constant adaptation and reinforcement of authentication methods with new approaches, especially new technologies. This article summarizes the set of analytical methods - classical and contemporary – that can be employed in the authentication of botanicals. Particular emphasis is placed on the application of untargeted metabolomics and chemometrics. An NMR-based untargeted metabolomic model is proposed as a rapid, systematic, and complementary screening for the discrimination of authentic vs. potentially adulterated botanicals. Such analytical model can help advance the evaluation of botanical integrity in natural product research.

Keywords

Botanical(s); Identity; Authenticity; Adulteration; DNA; Untargeted analysis; Metabolomics

1. Introduction

Botanicals (here defined as plants or parts of plants, but also lichens, fungi, and algae) used for medicinal purposes or health maintenance can be sold as plant raw materials or included in various preparations categorized as (traditional) herbal medicines or herbal products [1–4], herbal/botanical drugs [5], phytomedicines, natural health products [6,7], dietary supplements (DSs) [8], or food supplements [9], according to their final intended uses, and

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in compliance with prevailing regulatory requirements. The term botanical(s) is utilized here in lieu of plant raw material(s), or herbal raw material(s). Considering that botanicals are the building block of any commercialized finished products, determination of their authenticity is fundamental to supporting the purported effects and/or efficacy claims, as well as assuring the overall safety of any finished/commercialized product.

Regardless of their legal status, botanicals and their preparations play an important role in worldwide health care systems, and in many parts of the world they remain integral components of primary health care. In developed countries, herbal medicines/botanical DSs are increasingly utilized in complement with - as a first line of treatment for common ailments before considering the use of pharmaceutical drugs, or to address specific health concerns - or as alternatives to prevailing medical paradigms. The widespread consumption of botanicals, especially in the developed world, has significant economic impact, with the botanical DS market in the U.S. alone representing approximately \$7 billion for the year 2016 [10].

Quality assurance of botanicals is regulated primarily at the national level as a function of their legal status e.g., herbal drugs, traditional medicines, or food supplements [11,12,14]. This means that, in essence, the globalized botanical supply chain is not supported by a harmonized framework for the evaluation of botanical quality and authenticity. Incongruent statutory frameworks and the variety (and meaning) of quality control (QC) terminologies contribute to a certain level of confusion among consumers and international stakeholders alike [6,8]. This lack of a general agreement as to (a) the meaning of botanical authenticity and (b) the technical requirements related to certification of botanical quality increases the threat of botanical adulteration [15].

In academic research, the reproducibility and consistency of pre-clinical outcomes dedicated to evaluating the efficacy and safety of botanicals can be affected by use of insufficiently characterized material, for both crude samples and extracts, for which authenticity has not been assessed carefully [16]. In order to address this problem, the U.S. National Institutes of Health (NIH), via its National Center for Complementary and Integrative Health (NCCIH; formerly NCCAM) has developed policies (NOT-AT-05-003/004), which have been applied to NIH-funded biomedical research involving natural products since 2005. These have evolved into the current NIH Product Integrity Policy (PIP), which is available for consultation online at (<https://nccih.nih.gov/research/policies/naturalproduct.htm>). PIP stipulates that the identity and quality of botanicals, purified compounds, and other natural products utilized in NIH-funded research must be clearly established [17].

Reflecting increased public demand and scientific awareness, the number of studies dedicated to the authentication of botanicals and detection of adulterations has increased considerably in the past decade (Fig. 1). This observation parallels the popularization of techniques such as DNA barcoding, phytochemical profiling/fingerprinting, meta-bolomics and chemometrics being applied to botanical authentication processes. Regardless of the techniques employed, according to our literature survey both concepts of botanical authenticity and adulteration have shown interdependency.

The main objective of the present study is to propose a clarification of the integrated notion of botanical authenticity vs. adulteration, while emphasizing the relevance of a multilayered analytical strategy combining classical and contemporary techniques for the assessment of botanical authenticity. This article will not address problems relative to authentication of finished products, although many of the reviewed analytical methods will be applicable to them. Three key questions will be discussed herein: (1) what is/are the meaning(s) of botanical authenticity, and how does it relate to the problem of adulteration? (2) What is the contribution of targeted and untargeted analysis to the determination of both authenticity and adulteration? (3) What is the place of metabolomics in the QC toolset for the assessment of authenticity? In line with the third question, an untargeted metabolomic model will be proposed for the systematic screening of botanical samples. Ultimately, this article advocates for the dissemination and implementation of modern analytical tools/concepts that together can better address the determination of botanical authenticity with the stepwise discrimination of potential adulteration.

2. Exploring the meaning of authenticity and its relation to adulteration

2.1. The different facets of botanical authenticity

Terms are words or expressions that, in a specific context, are given specific meanings. Accurate and unambiguous terminology is fundamental to defining the concepts, terms and methodologies, collectively utilized in the inter-disciplinary activities conducted as part of botanical authentication. According to our literature survey, there is no official or unified definition of botanical authenticity, and, thus, a proposed definition of what makes a botanical “authentic” is somehow needed.

Authenticity involves (www.merriam-webster.com) “*being exactly as claimed, certified and certifiable, conforming to an original*”, with related words being “*validated, verifiable, correct, pure, unadulterated*”. In order to be conforming to an original, a botanical material should be identical, or share as many identical features as possible with that original.

The notion of identity is, therefore, inherently part of authenticity (Fig. 2). Botanical identity relies on a genetic or phenotypic delimitation, identification of the part(s) of the plant used, and/or an analysis of the characteristic chemical composition of a botanical, made under specified extraction conditions, that appropriately reflects its metabolomic profile at the time of harvest [4,18,19]. The phytochemical composition of genetically identical plant material can be affected by the composition of the soil, cultivation conditions, time of harvest, drying and extraction processes performed on the plant materials (Fig. 2). These parameters, with the exception of extraction, illustrate the concept of traceability.

Definition of authenticity, when applied to products such as food plants or processed foodstuffs, encompasses the certification of origin or supply chain transparency as well as the validation of composition in agreement with a certain mode of preparation. In the European Union (E.U.), the certification of origin contributes to the authenticity and economic value of food products, and, most importantly, ensures consumer safety [20,21]. Consequently, traceability is a primary contributor to authenticity.

The concepts “*pure, unadulterated*” stand in close relation to “*authentic*”. A fundamental condition of authenticity is, thus, the absence of adulteration or contamination. Hence, defining an adulterant, or the process of adulteration, is also fundamental to the establishment of authenticity.

2.2. Definition of botanical contaminations or adulterations

Merriam-Webster dictionary defines adulterate as “*to corrupt, debase, or make impure by the addition of a foreign or inferior substance or element*”. World Health Organization (WHO) guidelines for QC of herbal materials defines adulterated material as “*herbal material, an herbal constituent or other substance that is either deliberately or non-intentionally (through cross-contamination or contamination) added to an herbal material, herbal preparation, or finished herbal product*” [1]. Another definition is: “*to make impure by adding foreign, extraneous, poisonous, insanitary, or inferior substances/ingredients to a (food) product*” [22]. Adulteration is also described in great detail under title 21 (covering food and drugs) of the U.S. code of Federal Regulations [23].

A contamination, such as an environmental contamination, is an unintentional adulteration, and can result from cultivation in polluted area, misidentification or mishandling of the plant material, or careless harvesting methods that lead to the inclusion of undesired materials such as unwanted parts of plant material or other plant species. Phytochemical instability and degradation of plant material due to poor storage conditions can also lead to an adulterated botanical. Therefore, unintentional contaminations/adulterations generally result from poor QC measures within the supply and manufacturing chains [4,24].

Intentional adulterations are economically motivated and, unfortunately, their incidence is on the rise globally. They can occur through two different mechanisms: (a) complete or partial replacement of the initial plant material by an alternative non-certified, cheaper plant material, look-alike species, or another part of the plant, (b) addition of various amounts of unlabeled non-authorized chemical drugs, such as pharmaceuticals and synthetic derivatives, as a means of generating or increasing the claimed biological activity [25–29]. The so-called EMA (Economically Motivated Adulteration) is defined by the USP in the context of food fraud as: “*the fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser’s knowledge for economic gain of the seller*” [27,30]. Under U.S. statutes, botanicals are deemed adulterated and subject to law enforcement if they have been manufactured, packaged, and/or distributed without compliance to the current Good Manufacturing Practices (cGMPs) [8,22,23], or, in the case of a commercially-available packaged botanical or herbal product, if they are found to contain an ingredient not indicated on the product label [23,27,31].

2.3. Definition of botanical authenticity

We propose the following three-pronged definition of botanical authenticity: to authenticate a botanical is to validate (a) its traceability information, in terms of geographic origin, mode of cultivation and preparation; (b) its taxonomical and morphological identity; and (c) its (phyto)chemical composition contributing to its identity (chemotaxonomy), and supporting its purported health effect(s) confirming the absence of detectable contaminations or

adulterations, i.e. its purity (Fig. 3). Such a three-layer approach is also in agreement with fundamental QC steps specified under identity, composition/strength, and purity, in U.S. cGMPs for dietary supplements [8,22,31].

Authentication is a stepwise process of certification that includes the use of both validated methodologies, as discussed below, and of validated reference materials. Ideally and whenever possible, all steps leading to the validation of authenticity should be established according to official monographs or other validated guidelines provided by authoritative agencies such as Pharmacopeias (e.g., European Ph. EP, United States Ph. USP, Japanese Ph. JP), the World Health Organization (WHO), the Food and Drug Administration (FDA), and the European Medicine Agency (EMA), particularly the European Directorate of the Quality of Medicine and HealthCare (EDQM). Moreover, associations such as the American Herbal Product Association (AHPA: <http://ahpa.org/>), the American Herbal Pharmacopeia (AHP: <http://herbal-ahp.org/>), and the American Botanical Council (<http://abc.herbalgram.org>), also provide helpful guidance documents for a number of popular botanicals.

2.4. Reference plant materials to facilitate the assessment of authenticity vs. adulteration

As indicated above, to be considered authentic, a botanical material should ideally be certified conformed to an original, thus to a validated/certified reference material. In fact, assessment of the taxonomic identity, the phytochemical composition, and absence of adulterants/contaminants is facilitated by the use of certified reference plant materials (Fig. 3) representing authentic and vouchered botanical specimens of high quality [32,33]. For this purpose, independent science-based agencies such as pharmacopeias (e.g., USP, EP, JP), metrological institutes (e.g., the National Institute for Standard and Metrology NIST), as well as independent associations such as the American Herbal Pharmacopeia (http://herbal-ahp.org/bot_ref.htm), and other commercial providers, are now producing standard reference plant materials. This includes a variety of authentic phytochemicals (e.g., chemotaxonomical markers and other purified calibrants), for qualitative and quantitative analysis of botanicals. These Dietary Supplement/Herbal Reference Standards or Standard Reference Materials (e.g., NIST SRMTM, AHP-VerifiedTM Botanical Reference Materials) help both scientists and QC analysts in their assessment of botanical authenticity (Fig. 3) [4,32,34,35]. When sold as reference extracts, these materials represent botanical preparations characterized by a unique but well-established extraction process with defined concentrations of targeted markers. From a phytochemical assay perspective, the direct comparison of a sample of an as-yet indeterminate botanical to authentic reference standards (pure compounds, defined mixtures, and defined extracts) provides a high-quality evidence of botanical identity. However, factors related to the inherent phytochemical variability of some botanicals (a subject revisited in a later section) and the limited availability of authentic calibrants remains a challenge [28].

To conclude, an authentic botanical has a confirmed identity, a validated chemical composition, and is devoid of any adulteration (“*pure, unadulterated*”) in accordance to certified guidelines and by comparison with authentic reference materials whenever possible (“*conforms to an original, certifiable*”).

3. A three-step process for the assessment of authenticity

The multifaceted nature of botanical authenticity requires an integrated analytical approach [36], consisting of three main components (see Fig. 3): (step 1) documentation of traceability, which encourages supply chain transparency and helps identify risks pertaining to possible contaminations/adulterations [37]; (step 2) validation of botanical identity and integrity of the plant material or extract [38]; together with (step 3) the implementation of a variety of complementary phytochemical analyses to confirm the chemical identity and composition of the botanical, utilizing techniques that are also capable of detecting potential chemical adulterations [36]. Fraudulent contamination/adulteration of botanicals is often performed by recognizing and exploiting weaknesses in the analytical techniques typically used in botanical QC evaluation [37,39]. Therefore, it is important to assemble a strategically sound combination of efficient analytical tools that are capable of differentiating closely-related species, detecting unexpected biological and chemical contaminations and/or adulterations, and have the capacity to detect unusual/suspicious phytochemicals [36,38,40]. Various types of adulteration can be detected progressively through the combination of traceability documentation in step 1, which can provide insight into the identifying known environmental contaminants, morphologic and genetic identity tests in step 2, which can aid in the identification of biological contaminants, and phytochemical analyses in step 3 that can be applied to the detection of chemical adulterants.

3.1. Step 1: Traceability documentation and certification

3.1.1. Traceability guidelines and detection of environmental contaminants—

The primary category of well-known adulterants in botanicals are environmental contaminations such as heavy metals, pesticides, herbicides, mycotoxins, and (pathogenic) microorganisms, all related to plant growth in polluted soils, cultivation requiring the use of pesticides, harvest, and storage of the raw materials in unclean warehouses. Hence, failure in the control of supply chains and lack of traceability documentation can lead to the acquisition of poorly handled plant materials containing environmental contaminants [14,37,39]. A number of guidelines on Good Agricultural and Collection Practices (GACPs) are available that provide useful guidance on minimizing risks of contamination by reinforcing controls along the supply chain [4,24,41,42]. Documentation of location of cultivation/collection, time of harvest/collection and use of chemicals or other substances applied during production, such as fertilizers, pesticides, herbicides, fumigants, and growth promoters, is a fundamental requirement of GACPs [4].

Another series of guidance documents describe the methods to be used for the detection and quantification of environmental contaminants with their specifications. Such guidance documents are edited by the WHO [43,44], the herbal medicine compendium of USP (<http://hmc.usp.org>, see also USP general chapter <561>) [19,45], AHPA [46,47], and EMA (www.ema.europa.eu/docs) [4], and are also freely available online at the dedicated websites from each agency. A useful document edited by the AHPA compares the microbial limits set by the different authoritative agencies (WHO, USP, EMA) [47].

3.1.2. Certification of traceability and elemental analysis—Both market globalization and involvement of (multiple) distributors complicate the task of traceability documentation. Keeping the optimized determination of botanical authenticity in mind, there is a fundamental need for a change in the way botanicals are acquired, with the aim of enhancing transparency and controls throughout international supply chains [14,37]. All suppliers, including contract manufacturers and traders, are a critical link in the supply chain. While direct contact with local farmers or collectors is not always achievable, detailed documentation about the production, harvest, and sample processing should be accessible and provided by suppliers [4]. This would facilitate the authentication of purchased raw plant materials. Additionally, sharing information between partners along the supply chain facilitate the detection of suspicious trades [39]. Any gaps in the traceability chain and quality assurance programs are likely to be exploited for economic benefit [13,14,39].

Under U.S. regulation, cGMPs require documentation of traceability throughout the manufacturing chain, and DS manufacturers must assume responsibility for the accuracy of certificates of analysis received from suppliers through verification by their own internal testing [22,31]. The selection of specific verification tests (e.g, identity, traceability tests) is left to the discretion of the manufacturer [14,33]. Analytical techniques used to identify elemental composition of botanicals are perhaps most appropriate for corroboration of traceability information, with statistical comparison of multi-elemental composition been successfully applied in the determination of geographical origin or mode of cultivation. Therefore, such techniques are relevant to the assessment of authenticity of a number of food products and botanicals [48–51]. Among elemental analysis techniques Flame Atomic Absorption (AA) is the classical instrument, whereas Inductively Coupled Plasma, Optical Emission Spectrometry (ICP-OES) and ICP Mass Spectrometry (ICP-MS) are multi-elemental techniques with higher sample-throughput and better limits of detection than AA. All of the aforementioned techniques can also be used to detect the presence of heavy metals in botanicals (see also USP general chapter <233>) [45,51]. Particularly, ICP-MS has found increasing use to control the certification of geographical origin, and, thus, help documenting the overall traceability of any agricultural product. Moreover, phytochemical fingerprinting techniques in the context of metabolomics (see Sections 3.3 and 4 below) can also be utilized in evaluating the geographical origin of botanicals [40,52].

3.2. Step 2: Morphological & genetic identity tests

Methods used to determine botanical identity can be divided into two major groups: (a) analytical methods performed on plant raw materials, including microscopic and/or genetic identity tests, that are dedicated to the determination of botanical identity and the detection of biological contaminants, and (a) methods applied to extracts, essentially chemical in nature (and belonging to the field of analytical chemistry, that can be used for the (phyto)chemical verification of identity (chemotaxonomic analysis), the validation of the expected composition in line with the purported use(s) of the botanicals, and the detection of chemical adulterants [36,53,54] (see Section 3.3). Botanical identity tests should be specific enough to distinguish the correct plant species and plant part(s) from known and potential adulterants [43]. To that extent, morphological and DNA-based identity tests are performed

on botanical raw materials and have shown complementarity in their capacity to identify plant materials and discriminate them from their biological adulterants (fig. 4) [55,56].

3.2.1. Anatomical description—Taxonomic identity of botanical material has its roots in the anatomic/morphologic description of a plant phenotype. Macroscopic, microscopic, and, to a lesser extent, sensory analyses enable the identification and description of the part of the plant utilized for a certain therapeutic pharmacologic effect [18,36]. Detailed information regarding the importance of vouchered botanical samples and good identification practices can be found in a number of publications, including official guidance documents relating to GACP [24,38,41,42,57], as well as those proposed by botanical associations [58,59]. Technical methods and taxonomic keys for the identification of selected botanicals can be found in certified monographs from the authoritative agencies (EP, WHO, AHP and Herbal Medicines Compendium of USP) [54,60].

Macroscopic examination of a botanical is fundamental for the detection of foreign matter (e.g., soil, insects, molds), while microscopic analyses and the use of diagnostic keys helps to identify and discriminate the selected plant organ from other parts possibly present in the sample (e.g., presence of leaf epidermis in licorice root powder). Both types of analysis contribute to the detection of contaminations (i.e., foreign matter) (Fig. 4).

3.2.2. DNA barcoding: a complementary technique—Diagnosis of morphological features often cannot assure the identity of botanical materials that are rendered as fine powder. Other disadvantages to morphological identification methods include lack of character-based identification keys for certain plant life stages or the absence of specific microscopic features necessary for the distinction of closely related species [28,61,62]. Genetically-based identification techniques, notably DNA-barcoding [62,63], have become an additional/complementary means of determining the identity of botanicals (Fig. 4) [61,64]. DNA barcoding of botanicals involves the amplification of standardized regions of nuclear or chloroplast DNA to identify a plant species. Initially, the Consortium for the Barcode of Life (CBOL) recommended the use/amplification of several different regions (e.g., matK, rbcL, psbA-trnH, ITS2) to increase the accuracy of species identification and the capacity to differentiate closely related species [62]. Detailed information regarding DNA-based identification can be found in recent articles focusing on technical aspects [65,66] and/or discussing the utility of DNA-based techniques in the QC toolset for the identification of botanicals [55,56,61,63].

DNA-based identification methods can be limited by the quality/integrity of the DNA template, which might impact the amplification process on highly processed/extracted plant materials [55,63]. The amount of DNA amplified from a contaminant species does not necessarily correlate with its amount in the studied sample. Thus, it is possible to amplify the DNA of an impurity as opposed to that of the target botanical. Altogether, despite certain limitations, DNA barcoding has proven to be successful in detection and identification of botanical adulterants [55,67–69], including species sharing vernacular names or commoner and/or less expensive species. Such type of adulterations were reported to be frequent in the Indian [70,71] and Chinese [72] herbal medicine markets.

It is worth noting that both morphologic/microscopic analysis and DNA-barcoding techniques are qualitative analyses, and their diagnostic strengths rely on a careful representative sampling of plant material [43]. Macroscopically clean, morphologically or DNA-verified botanicals that are qualitatively deemed to be free of biological adulterants/contaminants should subsequently be further evaluated for their chemical composition.

3.3. Step 3: Phytochemical analyses

A number of excellent reviews have considered in detail the different steps and variety of techniques pertinent to phytochemical evaluation of botanicals [18,34,36–38,40,73]. Two general approaches to phytochemical analyses performed in the botanical authentication process consist of what are termed phytochemical profiling and phytochemical fingerprinting. Phytochemical profiling is employed when a targeted analysis is performed to evaluate a sample, focusing on a limited set of known chemical entities, i.e., phytochemicals or markers that can be detected, identified, and quantified. A phytochemical fingerprint, in contrast, reflects the overall chemical composition and complexity of a botanical under investigation. A phytochemical fingerprint is, thus, a “*totum*” of all compounds detected, both known and unknown, generated by untargeted analytical techniques [74,75]. In the scientific literature, the border between the terms “phytochemical profiling” and “fingerprinting” has become blurred. The terms targeted and untargeted should be employed to differentiate analyses dedicated to the identification and quantification of specific markers (targeted), as opposed to those techniques dedicated to producing a general overview of a sample composition (untargeted).

The quantification of specific markers in botanicals is usually performed through chromatography-based profiling methods, whereas fingerprinting approaches - notably spectroscopy-based analyses - are generally considered to generate an untargeted view of the sample composition and are preferably combined with statistical analyses or chemometrics (Fig. 5). Both approaches are useful (and complementary) for the determination of botanical authenticity and the detection of adulteration. A targeted analysis can define whether a botanical material contains the appropriate markers in a specified amount, and whether a known chemical adulterant may be present, and at what concentration. An untargeted analysis gives a snapshot of the overall chemical composition of the plant material, and favors the detection of unusual chemical features that could potentially result from adulteration.

3.3.1. Targeted & untargeted phytochemical analyses

3.3.1.1. Targeted analyses: Chromatographic techniques coupled with one or more detection systems, are currently the most widely used analytical systems for phytochemical identification of botanicals, and for both quantitative and qualitative evaluation of their phytochemical composition. Chromatographic methods are also widely utilized for the targeted detection and quantification of known adulterants. The most accessible and abundantly used chromatography-based techniques today are high performance thin layer chromatography (HPTLC), liquid chromatography (LC), including (ultra) high pressure systems ([U]HPLC) systems (coupled with ultra-violet (LC-UV), mass spectrometry (LC-MS), as well as other detectors), and gas chromatography (GC), typically coupled with MS

detection (GC–MS) [36,53,73,76]. Usually such analyses include the use of calibrants and/or certified reference extracts for the assessment of botanical authenticity. Targeted multi-marker identification and quantification reveals the overall uniqueness and phytochemical signature of a botanical, thus fostering its identification, a fundamental component of authenticity validation (Fig. 5). A series of validated methods or guidance documents, mainly for chromatography-based analysis of botanicals, have been developed and published by the Association of Official Analytical Communities (AOAC), as well as by national Pharmacopeias (e.g., USP, EP). These are more comprehensively documented in a number of reviews [34,40,77–79].

Targeted LC/GC-based analyses are typically implemented for the detection of analogues of pharmaceutical products (e.g., anti-hypertensive, anti-inflammatory agents, anabolic steroids) potentially added by unscrupulous producers to botanical materials and their preparations [25,80]. Reviews of different MS methods for the identification and quantification of the most frequent pharmaceutical adulterants fraudulently added to botanicals are available [25,80], including several that have underscored the frequent detection of synthetic adulterants in a wide range of Chinese and Indian herbal products [15,81]. Frequent examples of chemical adulteration of botanicals can be found in those products utilized as sexual enhancers and slimming products [82,83]. MS-based analytical methods are particularly useful in the detection of chemical adulterants that may be added in trace amounts to a sample, as well as to identify structural analogues of the considered synthetic adulterants. Methodologies for identification of chemical adulterants in botanicals have also been reported by HPTLC, capillary electrophoresis, and NMR techniques [25,84].

Typically, targeted LC/GC-based analyses are developed either for the phytochemical profiling and characterization of botanicals, or for the detection and identification of expected adulterants, but rarely for both at the same time. Targeted LC/GC-based analyses are generally optimized (e.g., type of stationary phase, UV wavelength, MS ionization conditions) for selected markers and/or suspected adulterants. As a consequence, there is an inherent limitation on the ability and degree to which targeted systems can readily identify unknowns, and this may constrain the potential detection of adulterants [85].

Exploitation of inherent limitations in analytical techniques used in targeted chemical profiling methods are illustrated by the example of *Ginkgo biloba* [86]. Phytochemical QC of *Ginkgo biloba* samples focuses mainly on the quantification of flavonol glycosides, and ideally of the chemotaxonomic terpene lactones (e.g., ginkgolides and bilobalide). Fraudulent addition of either flavonols (rutin and/or kaempferol) or cheaper plant materials rich in flavonols (e.g., *Sophora japonica*), have been reported as common adulterants in Ginkgo products [86]. In order to detect these types of adulterations, adaptations of certified analytical methods have been proposed for the QC of *G. biloba* [87,88].

In most cases, a combination of targeted analytical methods is necessary to confirm both the identity and the absence of *known* adulteration. However, because these analyses are targeted, they do not guarantee the absence of other possible adulterations. As stated in a recent research article: “*there is a need to develop or extend existing analytical approaches to identify unexpected adulterants*” [68].

3.3.1.2. Untargeted analyses & metabolomics: One explanation for the value of herbal medicines considers that botanicals may exert their purported health effect as a whole mixture of components that can act “synergistically” and/or via “polypharmacological” effects rather than by virtue of a few bioactive markers. This fundamental concept demands a more comprehensive understanding of phytochemical complexity. Metabolomics, the study of the chemical fingerprints that specific organismal cellular processes leave behind, provides a detailed look at the chemical profile of an organism. Small-molecule metabolomic analyses, through untargeted fingerprinting, have emerged as one of the methods suitable for the comprehensive chemical analysis of botanicals. In this context, untargeted metabolomic analyses entail the use of spectroscopic/spectrometric data to generate a comprehensive fingerprint through identification and quantitation of as many metabolites, i.e., phytochemicals or marker compounds, as possible [74]. Numerous authorities have recognized the value of fingerprinting analysis for the characterization of botanicals and their preparations, especially those for which active markers have not been identified, which continues to be the case for many botanicals [18,54,78,89,90]. Untargeted fingerprinting analyses are also useful when an entire botanical preparation is considered to be the active ingredient [90,91].

Qualitative/quantitative HPTLC, HPLC-based analyses, with hyphenated detection (UV and MS), as well as spectrometric (MS) and spectroscopic (IR/NMR) fingerprinting methods are typically utilized for untargeted fingerprinting. Spectrometric fingerprinting can be generated by flow injection MS (FIA-MS) techniques, which enable the production of complex MS spectra (i.e., mass spectrometric finger-prints) of botanical extracts, through direct injection to the MS source without LC-based separation [92–94].

Techniques utilized to generate spectroscopic fingerprints can be divided into two main groups: (a) vibrational spectroscopy, which includes infra-red (IR) notably Fourier Transform IR (FT-IR), near infrared (NIR), and Raman spectroscopy [95,96][97]; and (b) nuclear magnetic resonance (NMR) spectroscopy [98,99]. Each of the techniques cited above has its advantages and drawbacks in the field of botanical authentication, and thus, as is well documented in the literature, are complementary [92,95,96–100]. It is generally considered that comprehensive metabolomics can only be obtained by merging data from different analytical techniques or platforms. Due to their complementarity for the structure elucidation of compounds, both MS and NMR have emerged as the most widely used techniques in metabolomics.

The fundamental goal of metabolomics as applied to the evaluation of botanical authenticity - to gain as comprehensive a view of the phytochemical fingerprint of a species - requires an adequate diversity when sampling specimens of genetically/morphologically identical botanicals. This is necessary in order to understand the molecular signature(s) underlying macroscopic biological influences (e.g., species identity, chemotypes, geographical origins, cultivation conditions, extraction processes) [74,75,101–103]. Optimally, sampling from as wide a distribution of the species under investigation as possible in order to establish the natural range of inherent chemical variation between populations helps to generate a dataset containing the best representation of fingerprints from authentic botanicals, and gives a sense of natural phenotypic chemical variation found within and between populations of a

species. Once established, these comprehensive fingerprints can then be used to facilitate the rapid detection of unknown chemical features - resulting from chemical deterioration, unknown types of contamination, and the addition of foreign species or chemical adulteration.

As pure spectrometric FIA-MS, and spectroscopic IR or NMR analyses do not involve physicochemical separation of compounds, these techniques generate “complex unbiased phytochemical snapshots or fingerprints” of botanical materials [75,95,98,100]. In order to objectively compare different botanical samples with a set of reference spectra from authentic botanicals, and thus, to eventually detect potential adulterants, multivariate statistical analyses called chemometrics (statistical analysis of chemical data) are generally employed.

3.3.2. Chemometrics for the determination of authentic and adulterated botanicals—Chemometrics combines multivariate statistics and mathematical modeling of chemical data (profiles, fingerprints) to simultaneously analyze and compare multiple measurements and identify relationships within them. In other words, chemometrics facilitates the identification of chemical patterns (or features) that enable the classification and/or comparison of botanical samples in terms of their chemical similarity (or difference). Therefore, chemometrics is particularly useful for QC applications, including the discrimination of authentic and adulterated botanicals. Chemometrics help reducing the complexity and dimensionality of the produced phytochemical data, and analyze the relationship between variables (e.g., chemical shifts, signal intensities, m/z, absorption wavelengths) and samples (e.g., botanical extracts). Result interpretation is assisted by the graphical representations of mathematical outcomes [104,105]. The wider implementation of chemometric methods has resulted in the availability of an increasing variety of chemometrics software solutions [90,104,105–107].

In practice, chemometrics as part of the QC toolset entails: (a) the acquisition of several phytochemical profiles/fingerprints from multiple morphologically/DNA-identified botanicals and reference plant materials; (b) data processing to help remove technical artifacts and background noise (dependent on the considered analytical technique); (c) the subsequent statistical analyses of extracted chemical data for comparison, classification, and for the generation of a model defined by control limits that can be applied to represent the parameters of authenticity for the set of identical botanicals sharing similar variances; and, finally, (d) the validation and improvement of the generated classification or models [90,103].

3.3.2.1. Choice of chemometric analysis for the detection of known vs. unknown adulteration: As already described by Brereton and summarized by Gad HA et al., chemical pattern analysis, in chemometrics, is generally said to be either exploratory, unsupervised, or supervised [104,108,109]. Exploratory analysis does not require a prior knowledge of sample class or composition (e.g., species identity, or group of identical species cultivated in one single region), and enables the clustering of samples according to their acquired chemical fingerprints/profiles. Such analysis helps identifying patterns that could be used subsequently for classification or modelization. In unsupervised pattern

recognition, the analyst does not assign sample class (similarly to exploratory analysis). Unsupervised analysis is used to detect major similarities between sample spectra, leading to their clustering without prior knowledge on their chemical composition. Supervised methods utilize information gathered notably from exploratory and unsupervised analyses to build a classification model. Supervised chemometric approaches require more stringent validation to evaluate the accuracy of the generated models.

The most widely applied forms of chemometrics for the phytochemical comparison, differentiation and classification of botanical samples are exploratory and unsupervised methods utilizing statistical functions like principal component analysis (PCA) and hierarchical cluster analysis (HCA), as well as supervised analyses with soft modeling of class analogies (SIMCA), and partial least square discriminant analysis (PLS-DA). For the analysis of samples with unknown chemical composition and the detection of unknown (chemical) adulteration, exploratory PCA combined with SIMCA are particularly useful. Supervised statistical classification method such PLS-DA are particularly appropriate for the construction of models dedicated to the identification of known adulterations, notably in the context of targeted analysis [92,110]. More information regarding the implementation of metabolomics and chemometrics for the phytochemical comparison/classification of botanicals, and the evaluation of authenticity can be found in various reviews [104,110–113].

3.3.2.2. Examples of untargeted metabolomics applications for authenticity

assessment: With the help of chemometrics, genetically and/or morphologically determined botanicals can be classified and differentiated according to the multiple factors that affect their phytochemical composition and, thus, the definition of authenticity. As such, morphologically identical botanicals can be differentiated based on their chemotypes, material preparation, or traceability information (geographical origin, mode of cultivation). Additionally, certain models such as SIMCA can assimilate a set of authentic botanicals by considering all their possible phytochemical variances within defined statistical limits.

Chemometric analyses provide powerful tools to analyze complex datasets, and the scientific literature is rich with examples showing the successful implementation of metabolomics and chemometrics for botanical QC [90,104,106], including the following: classification and chemical differentiation of closely related botanical species or cultivars [94,114][115–117]; identification of geographical origins of samples as demonstrated with green tea [118], various medicinal herbs [95] such as *Panax ginseng*[119] and food plants [120]; and differentiation of botanical preparations as exemplified with feverfew [121] and *Scutellaria lateriflora*[93] products.

Evidence for the value of chemometrics in detecting adulteration is also well represented in the literature. Examples include analysis of a series of untargeted chromatographic and spectroscopic fingerprints to identify adulterations characterized by total or partial replacement of the purported plant raw material with that of another species [119,122,123], by addition of unwanted plant parts of the same species [124,125] or chemicals/pharmaceuticals [110,126]. LC-UV based spectral fingerprints, combined with SIMCA analysis, and ¹H NMR fingerprinting methods combined with PCA, were utilized in separate

experiments for the assessment of *Ginkgo biloba* authenticity and the simultaneous detection of potential adulteration that resulted from admixing with other plant species or chemical spiking [87,88]. FT-IR [124] and NMR-based [125] supervised chemometric models (PLS-DA) were able to detect known botanical adulterants in saffron spice, with detection limits between 5 and 20% w/w for IR and 20% w/w for NMR-based models. Likewise, the combination of ¹H NMR fingerprinting with PLS-DA enabled the detection of defined botanical admixture/adulteration of *Panax ginseng* [119] phytochemical degradation during storage of saffron [127], and chemical dyes in curry, turmeric, and paprika spices [126]. Applying SIMCA models to metabolomic fingerprints revealed adulterated licorice [114], Ginkgo [87], and American ginseng [128]. A parallel screening of oregano by untargeted FT-IR with chemometric analysis, and a targeted high resolution MS analysis was conducted in order to demonstrate that both approaches are complementary, and led to congruent results with regards to the determination of authentic vs. adulterated oregano samples [129].

4. Proposed metabolomic screening for the discrimination of authentic vs. potential adulterated botanicals

Metabolomics has demonstrated its utility for the assessment of botanical authenticity, yet its implementation remains sporadic in the greater botanical arena, being limited to academic or governmental research settings. Several limitations need to be addressed in order to expand the use of untargeted metabolomics and chemometrics for the systematic evaluation of botanical authenticity and the recognition of adulteration. As reviewed by Esslinger et al. [112] in the context of food QC, several key technical limitations of these techniques are relevant to the QC of botanicals, and some of them are briefly considered in the following, before going into the detail of a practically feasible meta-bolomic screening approach.

4.1. Limitations of currently used metabolomic approaches

In order to accurately represent the phytochemical composition and variation between different samples of supposedly identical herbal materials, a certain number of representative botanicals should be collected from diverse well-documented sources and/or diverse botanical populations [28,33,36]. Such reference botanicals must be sufficiently representative to integrate all expected botanical/phytochemical variances [110], although the minimum number of required samples remains unclear. No specific guidance on this issue can be found in the literature, even though it is clear that the number of samples is critical for the establishment of statistical significance and representative data set. The choice of sample size is a critical task in part because the number of samples depends on a variety of factors, including the botanical species in question, the number of possible varieties, their sourcing modalities, geographical distribution, cultivation conditions, or even possible variations due to endophytic micro-organisms [92].

Other limitations are related to technical/instrumental parameters. It is necessary to regularly control the calibration of any instrument in order to obtain comparable results through times. Ideally, in LC-based profiling/fingerprinting analyses both the reference materials and the samples themselves should be acquired together in the same batch or sequence analysis, leading to the progressive consumption and eventual depletion of the reference material(s)

[92]. In the particular case of IR, the quality of generated spectra depends on both the physical state of the samples as well as the environment of data acquisition [97,100]. Both IR and MS fingerprints are instrument and laboratory dependent, and in most cases the results obtained in one laboratory are not easily transposable to any other. Published results notably in terms of acquired data (raw and processed), collected from a specific instrument cannot necessarily be utilized directly by another laboratory to assess botanical authenticity or detect adulterations. Despite problems associated with the stability of measurements over time, across instruments and laboratories, the value of metabolomics in botanical QC remains obvious [102,104,106]. To that extent, inter-laboratory comparison/validation studies as well as uniformity of the exchangeable data are needed [112,113]. Interestingly, some inter-laboratory validation studies have been performed with NMR.

A study published by Ward JL et al. has demonstrated that comparable inter-laboratory NMR spectra could be obtained by standardizing the NMR measurements from instruments of the same type and vendor, provided that sample preparation is also standardized [130]. Another study confirmed the robustness of NMR fingerprinting by comparing, through chemometrics, the ^1H NMR spectra of low bush blueberry leaf extracts acquired by different users in different laboratories, even on instruments with different probe types [116]. Provided that sample preparation and spectral acquisition are standardized, NMR-based fingerprinting can deliver reproducible spectra over time and in different laboratory environments. Therefore, NMR has been suggested as a technique of choice for the reproducible acquisition of spectral fingerprints, enabling the comparison of spectra of new botanicals with archived authentic spectra.

4.2. Proposed NMR-based metabolomic screening of botanical authenticity

The following approach proposes the use of NMR-based fingerprints for the development of chemometric models of authentic spectra that can be utilized for authenticity screening of newly acquired botanicals. We propose herein to consider the implementation of ^1H (or ^{13}C) NMR-based metabolomic fingerprinting at the final stage of the QC process, once the identity of the botanical material has been validated by morphologic/DNA analysis, and/or targeted phytochemical analyses as specified in Section 3.

4.2.1. Outline of ^1H and ^{13}C NMR-based metabolomic botanical screening—

NMR-based metabolomic fingerprinting for the authentication of plant materials involves four main steps that eventually lead to the determination of botanical authenticity (Fig. 6). The process starts with a set of well-identified, traceable raw botanicals, which are extracted using a standardized process (Fig. 6A). Subsequently, ^1H (and/or ^{13}C) NMR fingerprints are acquired using standardized operating procedures adapted to the respective NMR instruments [130]. The pool of acquired spectra is processed and archived to constitute a database (ideally open access) of reference spectra of authentic botanicals (Fig. 6B). All reference spectra are then subjected to chemometric analyses. Following statistical validation steps and/or calibration, a model of authenticity is generated. Such a model (e.g., SIMCA) represents a group of authentic plant materials defined by identical phytochemical features that may vary as a function of their geographical origin, cultivation/harvest conditions, chemotypes, etc., with a statistically-modelled range of acceptable chemical

variability. This four-tier metabolomic process leads to the production of a chemometric model that can be defined as a digitized model of botanical authenticity, constructed with NMR fingerprints and specified by statistical limits [110]. This model can be employed subsequently to rapidly screen for authenticity/adulteration or even phytochemical deterioration of newly acquired botanicals, provided that they are prepared and their fingerprints are acquired according to standardized procedures (Fig. 6C).

Newly acquired NMR fingerprints are then tested against the set of reference spectra utilizing the chemometric model (e.g. SIMCA). When classified within the statistical limits of the model, the NMR spectra, thus the tested botanical, would be determined as reasonably authentic (Fig. 6C sample #1). Inversely, the acquired fingerprints of samples classified outside the set of reference spectra (i.e. outliers, Fig. 6C, sample #2), thus outside the statistical limits of the model, would be considered as non-authentic, thereby suggesting a potential adulteration or chemical deterioration. Under this model, potentially adulterated botanical samples would be further investigated by complementary analytical methods, in order to confirm the presence or absence of possible adulterants.

4.2.2. Challenges & limitations of NMR-based metabolomic botanical screening

—One of the main challenges of the proposed NMR-based metabolomic screening lies in the acquisition of spectra from as many authentic botanicals as possible, including also as many adulterated botanicals as possible to further validate the statistical model. To become feasible, the proposed screening approach calls for international inter-laboratory collaboration and the development of an open access centralized database of archived ^1H (or ^{13}C) NMR spectra of botanical reference materials (e.g., as crude extracts) deemed authentic according to the analytical methods presented in Section 3. This approach will have the ability to complement traditional QC methods. The archived standardized NMR spectra will represent a digitized set of authentic botanical references that can be utilized and improved as additional insights are gained about the respective botanicals.

A second challenge is related to the comparatively (vs. MS) low sensitivity of NMR [75,98], which might limit the capacity to detect chemical adulterants present in trace amounts. However, independent studies have demonstrated that low levels (e.g., limit of detection 6.7 mg/kg) of Sudan dyes adulterants in spices could be detected by ^1H NMR analysis [131,132].

The ^1H NMR spectrum of a botanical can be understood as the superimposition of all NMR spectra of all individual compounds present in the sample [75]. Thus, the capacity to correctly detect adulterated/-deteriorated samples will not only depend on the quantity and the quality of reference spectra, but also on the capabilities of the proposed statistical analyses.

Other notable, but surmountable, practical challenges that might affect the feasibility of the NMR-based chemometric approach are the availability of NMR instrumentation and incompatibilities between raw NMR formats generated by instruments from various manufacturers. The harmonization of NMR formats between various manufacturers is a

work in progress, with a notable initiative being spearheaded by the Allotrope Foundation (<https://www.allotrope.org/>).

4.2.3. General advantages of NMR-based metabolomic botanical screening—

The proposed NMR based metabolomic model for the screening of botanical authenticity has a number of general advantages: (a) it is not associated with the depletion of reference botanicals and reference phytochemicals over time, as the reference spectra need to be acquired only once, and NMR is a non-destructive technique; (b) it will have the advantage of integrating as many botanical/phytochemical variances as possible, provided the model is built with efficiently sourced raw plant materials; (c) as ^1H NMR fingerprints offer an unbiased overview of phytochemical composition with however a favorable detection of the most abundant compounds, it will facilitate a comprehensive phytochemical characterization of botanicals; (d) the combination of phytochemical fingerprints with statistical analyses also facilitates the rapid detection of unusual profiles to identify chemical features of adulteration. Furthermore, this model could be optimized over time, with progressive acquisition of reference spectra from authentic, but also adulterated, botanicals adding to the library of reference spectra. Documentation of traceability for each integrated authentic botanical will support the interpretation of metabolomic classification, thereby promoting and/or corroborating supply chain transparency.

A collaboration between food industry and an NMR manufacturer led to the development of an NMR-based screening method for food products that is dedicated to the evaluation of authenticity and the detection of potential adulterations in fruit juice, wine, and honey [133,134]. The methodology relies on a model comparable to what is proposed above, where the ^1H NMR spectra of new samples are compared to a group of reference spectra obtained from various samples, e.g., juices, wines, honeys from different geographical locations, through the implementation of multivariate data analysis and chemometric models. The establishment of this analytical tool supports the feasibility of the proposed NMR-based metabolomic screening of botanical extracts for the rapid evaluation of authenticity and/or detection of adulteration.

As indicated above in the entire Section 3, it is highly unlikely that one single technique can enable the determination of botanical authenticity. Such NMR-based authenticity screening is proposed to serve as a complement to other currently implemented QC methods. This proposed model holds the potential to rapidly identify botanical samples with unusual chemical features that could result from adulteration requiring further evaluation by complementary analytical techniques.

5. Conclusions

This perspective has presented an overview of the integrated assets – analytical tools, concepts, and controls – that are necessary to authenticate a botanical in the face of threats posed by adulteration, especially those forms that exploit vulnerabilities in the QC toolset [13,39]. The meaning(s) of botanical authenticity and its inherent relationship with adulteration, the contribution of analytical techniques to the total effort, and the place of

metabolomics in the QC toolset were explored, along with the presentation of a proposed NMR-based un-targeted metabolomic model.

5.1. Authenticity assurance and adulteration management are linked

We propose that an authentic botanical should possess a confirmed identity, proven chemical composition, and be devoid of any adulteration (“*pure, unadulterated*”), in accordance with accepted (pharmacopeial) guidelines and by comparison with authentic reference materials, whenever possible (“*conforms to an original, is certifiable*”). Thus, validating the authenticity of botanicals also means confirming the absence of potential adulterations, whether classified as unwitting environmental contaminations or economically motivated adulterations. Authentic botanicals are by definition non-adulterated.

The assessment of botanical authenticity (i.e., “*being exactly as appears or as claimed, validated, pure, unadulterated*”) goes beyond the mere determination of morphologic and genetic identity. Thus, it also includes the documentation of traceability of the considered botanicals, which promotes the awareness for potential adulterations along the supply chain, as well as the evaluation of their qualitative and quantitative phytochemical composition. The complementarity of the different analytical steps that lead to the validation of botanical authenticity ultimately enables the detection of practically relevant types of contamination and/or adulteration, in a stepwise manner.

5.2. Cutting-edge authentication methods help counter adulteration

The ever-increasing sophistication of willful adulteration requires a permanent awareness of the existing risks, and dictates the modernization of analytical methodologies enabling the assessment of botanical authenticity. This condition necessitates permanent technical training and specialization of the teams responsible for botanical QC. To this end, the American Botanical Council (ABC), the American Herbal Pharmacopeia (AHP), and the University of Mississippi’s National Center for Natural Products Research (NCNPR) have recently developed the NCNPR-ABC-AHP botanical adulterants program (www.botanicaladulterants.org), which aims at educating the stakeholders about the risks of adulteration, maintaining awareness, and proposing guidance for the detection of adulteration. The program regularly publishes Botanical Adulterants Bulletins as well as Laboratory Guidance Documents, and participates in educational programs/workshop on the adulteration topic. Another useful tool in this regard is the USP Food Fraud Database (www.foodfraud.org) [14], a regularly updated repository that increases the knowledge on existing adulterations gathered from reported frauds, while proposing various analytical methods for their detection.

5.3. The integrated assets approach

Efficient QC for the assessment of authenticity entails a multi-disciplinary, integrated analytical approach with expertise in botany (taxonomy, macroscopic and microscopic analyses, molecular biology), and analytical (phyto-)chemistry [36,61]. No single analytical method can address the determination of botanical authenticity on its own. With regard to the evaluation of phytochemical composition, targeted chromatography-based analyses are the current *de facto* standard for the multi-constituent identification and quantification.

However, the targeted nature of such analytical methods intrinsically limits their overall fitness for the purpose of performing the detection of unknown/unexpected adulterants. The concurrent use of both targeted and un-targeted analytical tools not only builds a more integrated set of analytical authentication assets, but also lowers the risk of exploitation by willful adulteration. Likewise, the holistic definition of botanical efficacy demands a more comprehensive, ideally unbiased, analysis of phytochemical composition.

Collectively, these observations call for a more comprehensive, ideally untargeted, (phyto)chemical analyses of botanicals, which ultimately require the implementation of metabolomic profiling/finger-printing methods utilizing notably IR, NMR, and MS techniques. The statistical approaches (chemometrics) behind metabolomic analyses facilitate the compilation of multiple phytochemical variables that enable the classification of authentic vs. non-authentic botanical samples. Untargeted spectrometric/spectroscopic analyses are preferable as they have the potential to detect unknown chemical features potentially resulting from adulteration. Hence, untargeted analytical methods combined with chemometrics have the potential to make the QC of standardized botanical preparations more effective as they chemically evaluate the authenticity of botanicals and can simultaneously capture potential adulterations, including those not yet known to the scientific community.

5.4. The value of NMR-based metabolomic fingerprints

Based on a comprehensive review of the literature and in-house experimental evidence, the authors propose an NMR-based metabolomic approach for authenticity evaluation of botanical samples through statistical (chemometric) comparison of NMR fingerprint spectra. Owing to the robustness of NMR as a relative primary analytical technique, the value of NMR-based metabolomic models can be potentiated through international inter-laboratory collaboration. Especially helpful would be the development of a unified collection of NMR spectra of authentic botanicals, which could serve as a digitized set of authentic spectra, and subsequently be integrated into statistical analyses for the definition of a chemometric model of botanical authenticity. The proposed NMR-based metabolomic screening model for raw botanicals and extracts offers dual benefits: on one hand, it integrates the multiple factors that define the authenticity of botanicals through the use of statistical models; on the other hand, it facilitates the detection of unusual chemical patterns that could result from sample deterioration or adulteration.

5.5. Botanical authenticity in the international marketplace

While technically feasible, the implementation of metabolomic tools for the assessment of botanical authenticity continues to face broader implementation hurdles, which should be considered carefully: (a) standardization of metabolomic analysis in botanical QC, including sample preparation and data acquisition; (b) the implementation of inter-laboratory collaboration, ideally involving science-based authoritative agencies and associations, as a means of achieving representative coverage (geographical distribution) of botanical specimens; and (c) establishment of an infrastructure that allows exchange and re-use of data and results [112]. Considering that the aim of botanical QC is to ensure and protect human health, the major effort of the required concerted initiative, comparable to the Metabolomics

Standards Initiatives (MSI) [135], is justified, but also requires international coordination to become real and effective. Considering the paramount importance of authenticity and adulteration in the contemporary global botanical marketplace, now is the time to bridge the gap between the international botanical/herbal DS industry, regulatory agencies, pharmacopeial authorities and academic researchers, in order to promote the implementation of cutting edge techniques/approaches into widely practiced botanical QC measures, for the benefit of enhancing the reproducibility, efficacy, and safety of botanicals.

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Fig. 1.

Publication trends extracted from PubMed (status: October 2017) and related to the following themes: botanical authenticity, botanical adulteration, metabolomics, and DNA barcoding techniques in relation to the concept of botanical authenticity. The extracted publication counts highlight the strong interconnections between the notion of botanical adulteration and authenticity and the new/modern approaches for the assessment of botanical authenticity. This article proposes to evaluate the relationship between botanical adulteration and authenticity, while assessing the role of DNA barcoding and metabolomics/chemometrics in the QC process to discriminate authentic and adulterated botanicals.

Fig. 2.
Parameters affecting the determination of botanical authenticity.

QUALITY CONTROLS

new botanical sample

Adulterated if...

Fig. 3.

Interconnections between the concepts of adulteration and authenticity. Certification of traceability, determination of botanical identity, and description of phytochemical composition are part of the QC measures. When designed wisely, these different QC steps enable the progressive detection of different types of adulteration. Hence, the concepts of botanical authenticity and adulteration are two sides of the same coin, the rim of which represents the set of analytical methods utilized in the botanical QC toolset. The use of certified reference materials can help in the analytical process towards the validation of botanical authenticity.

Fig. 4.

Complementarity between morphological (A) and genetic-based (B) botanical identification methods. (A) Morphologic analyses are suited for the identification of raw plant materials (identity of species and plant part used), provided that the physical aspects of the investigated sample (entire plant parts or powders) enable the observation of characteristic features. (B) Genomic-based identification methods are particularly suitable for species identification when morphologic analyses are impossible or not specific enough. Species identification by genetic-based methods is limited by the quality/integrity of the DNA template. Both morphologic and genetic-based analyses can help in the detection of biological adulterations.

Fig. 5.

Targeted vs. untargeted analysis for the determination of authenticity and the detection of adulterations. Chromatographic analyses are mostly targeted and suitable for the multi-constituent standardization of preparations, especially when known compounds are responsible for a certain health effect. These techniques are also utilized for the detection and quantification of known adulterants. In line with the holistic notion that botanical preparations exert biological health effects as a whole, non-targeted analyses have emerged as a complementary tool as they offer a more comprehensive overview of the (phyto)chemical composition. Non-targeted spectroscopic approaches are more likely to assist in the detection of unusual chemical composition that can be related to sample degradation and the presence of adulterants. Both targeted and non-targeted analyses are necessary for the phytochemical assessment of botanical authenticity.

Fig. 6.

NMR-based metabolomic model for the screening of botanical authenticity and rapid detection of potential adulteration. (A) The principal step towards the development of metabolomic screening model calls for inter-laboratory collaboration in order to acquire as many (ideally: authentic) botanical reference spectra as possible. This step requires the implementation of standard operating procedures (SOP) for sample preparation and data acquisition. (B) All the acquired spectra are deposited in an open access repository where they can be further processed and compared by chemometric analyses. (C) Different laboratories can compare their own spectra from new botanical samples (prepared and acquired according to the SOP) to the spectra in the database. The newly acquired spectra can be tested against the statistical model of authentic spectra so as to quickly and objectively evaluate their authenticity defined by statistical similarities when compared to a set of referenced/digitized spectra (i.e., samples within the limits of the chemometric models sample #1). The same process is capable of detecting major chemical differences that lead to their classification outside the model and conclusion as being non-authentic or adulterated (sample #2).