

Decision-Oriented Quality Assurance of Herbal Medicines: An Integrated Framework for Authentication, Standardization, and Safety

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ABSTRACT

Herbal medicines are made up of many different chemical and biological substances, so their quality can change depending on the plant's identity, where it is grown, climate, harvesting stage, processing, storage, and manufacturing methods. Because of this complexity, their quality cannot be checked by testing only one compound or using one method(1,2). Traditional methods are used to identify the correct plant, while chemical, physical, chromatographic, and spectroscopic tests, along with DNA-based identification and contaminant testing, are used together to make sure the herbal medicine is safe, pure, and of good quality.(1–4)

This critical review aims to provide a decision-making framework in which five quality control questions pertain to different levels of evidence. HPTLC and HPLC fingerprints offer routine approaches to chemical profiling and marker analysis, whereas more comprehensive analyses of chemical compounds can be achieved by means of LC–MS, GC–MS and NMR. Botanical identification by DNA barcoding is orthogonal to establishing chemical quality control and safety (3–7). Metabolomics, chemometrics, hyperspectral imaging, machine learning and new portable spectroscopy techniques are also discussed with regard to their potentials and limitations, opportunities for validation and regulatory relevance (8–11).

Unlike many previous reviews, this article integrates information on analytical technologies according to the specific quality control decisions rather than presenting an inventory of relevant approaches. Emphasis is placed on the need to interpret findings in the context of particular quality attributes because any single technology or combination of methods may not be suitable for all stages of manufacturing (1–4). Particular areas of research include the need for reference materials and spectral and DNA databases, opportunities for external validation of metabolite identification and machine learning algorithms, the importance of establishing inter-laboratory reproducibility and comparable acceptance criteria, and regulatory relevance of different analytical technologies for authentication (4,8–11).

KEYWORDS: Herbal medicines; quality assurance; authentication; standardization; adulteration; HPTLC; HPLC; DNA barcoding; metabolomics; chemometrics; machine learning; regulatory quality control.

1. INTRODUCTION

Herbal medicines are important healthcare products and are available in many forms, such as raw herbs,

extracts, phytotherapeutic products, nutraceuticals, and finished medicines. Their quality can change due to factors such as the type of plant, environment, farming methods, processing, and storage.(1,2) Authentication is very important because herbal medicines can be adulterated, replaced with other plants, or wrongly identified, which can make them ineffective or unsafe. Studies have found that some herbal products may contain different plant species, missing ingredients, or unwanted organisms that cannot be identified by simply looking at the product. Therefore, more than one testing method is needed for complete authentication. Identifying the plant alone cannot confirm that no other plant parts have been added, while chemical testing alone may not detect all plant species or contaminants. Additional tests are needed to check for harmful contaminants such as bacteria, fungi, pesticides, and heavy metals(1,3,5). This underscores the need for rational quality assurance, using complementary methods to demonstrate different aspects of quality.

Previous reviews have discussed various conventional and contemporary analytical techniques used for authentication, DNA barcoding, and phytochemical chemometrics and multivariate data analysis (2,3,6,8). More recently, authentication using DNA-based orthogonal methods and the application of state-of-the-art analytical techniques in combination with machine learning have been reviewed [9,10]. Thus, a further descriptive review of the methods, their principles, advantages, limitations, and applications would be largely superfluous. Therefore, this review explains the strengths and limitations of different testing methods by showing what each method can identify, what it cannot identify, and what additional tests or information may be needed to get a clear and reliable answer.

2. REVIEW METHODOLOGY

This article is a critical review and not a systematic review or meta-analysis. The researchers collected information from sources such as PubMed/MEDLINE, publisher websites, and WHO databases. They searched these sources using keywords related to herbal medicines, quality control, authentication, adulteration, and different testing methods such as HPTLC, HPLC, LC–MS, GC–MS, DNA barcoding, metabolomics, chemometrics, machine learning, and hyperspectral imaging.

Found papers were grouped according to the foundational principles that were well-established by the time of the review and the more recent advances in analytical and computational methods (2–10).

Articles with specific goals related to identity, adulteration, chemical profiling, standardization, safety evaluation, analytical authentication, chemometric analysis, or new approaches to data-driven quality control of herbal medicines, materials, or products were included. Articles of special interest were reviews, methodological studies, reports focused on commercial products, and quality control guidelines. The rationale for this approach was that it can best address the objective of synthesizing the methodological evidence while focusing on the opportunities for translation into practice, rather than assessing a treatment effect.

3. WHY HERBAL MEDICINE QUALITY REQUIRES MULTIPLE TYPES OF EVIDENCE

Herbal materials comprise different constituents that vary in concentration depending on the species, chemotype, place of origin, agro-climatic conditions, stage of plant growth and harvesting techniques. Therefore, there is a need to identify more than one compound as a marker of a biochemically complex material with multifactorial activities or when the marker is not specific to the stated species or genus (2,3).

The commercial significance of herbal medicines and the associated problems with adulteration,

contamination, and misidentification have been underscored by the chemical authentication of 2,386 commercial herbal medicines from 37 countries. The results revealed that 27% of the samples were adulterated and 29% lacked the purported botanical ingredients, while the remainder had phytochemical inconsistencies. This finding suggests that there is a need to develop quality control processes that go beyond establishing identity to include chemical profiling, as the two are not interchangeable (4).

A suitable and cost-effective methodology for herb identification is dictated by the quality attributes sought and the nature of the herbal matrix. Thus, a visual inspection of a milled or dried herb is unsatisfactory compared to a macroscopic and microscopic examination of the crude drug. Similarly, chemical fingerprintings and/or molecular approaches may be required to establish the identity of a processed herbal preparation since anatomical features may be absent or compromised during processing (1,4,5). In the case of multiple ingredients, the issue is further complicated by the fact that a given analytical methodology may not be adequate in demonstrating the quality attributes of all the constituents.

3. DECISION-ORIENTED QUALITY-ASSURANCE FRAMEWORK

The framework proposed here encompasses five quality questions: (i) identity - whether the declared botanical material is present; (ii) purity/adulteration - whether substitution, admixture, or extraneous material is present; (iii) chemical consistency - whether the product has an appropriate chemical profile or marker specification; (iv) safety - whether relevant biological and chemical contaminants are controlled; and (v) reproducibility/traceability - whether the quality decision can be reproduced across batches, suppliers, and laboratories (1,2,4).

These questions will not have a unique analytical solution. Botanical examination and DNA-based methods primarily address authenticity and identity; microscopy, chromatography, and spectroscopy can provide supporting evidence on botanical identity and chemical profile; appropriate targeted instrumental methods are required to address specific contaminants; and classification algorithms based on chemometrics or machine-learning approaches may be deployed when adequate reference data sets are available (3,5,6,8,10).

Thus, the general proposition for an orthogonal-evidence approach is that specific methodologies should be applied that have different error modes so that the combination provides greater confidence than any single method alone. DNA barcoding provides different evidence from traditional morphology-based authentication, and chemical profiling provides different evidence from DNA-based authentication; each alone may be considered insufficient analytical support for a quality decision (5,9).

Table 1. Quality questions mapped to analytical evidence

Quality question	Primary evidence	Complementary evidence	Major limitation	Recommended interpretation
Identity	Macroscopy, microscopy, DNA barcoding	Chemical fingerprint	DNA may fail with degraded DNA; chemical similarity may be non-specific	Use at least two orthogonal evidence types when risk is high (1,5,9).

Purity/adulteration	Microscopy, HPTLC/HPLC, DNA methods	LC-MS, spectroscopy, chemometrics	Closely related substitutes and processed matrices can complicate interpretation	Choose methods according to the suspected adulterant and matrix (4-6).
Chemical consistency	HPTLC/HPLC fingerprint, marker assay	LC-MS, NMR, metabolomics	Reference profile and validated acceptance criteria are required	Assess both pattern similarity and quantitative specifications (2,3,8).
Safety	Microbiology, mycotoxin/pesticide/elemental analysis	Risk-based targeted testing	Contaminant profile varies with material and supply chain	Apply contaminant-specific validated methods (1).
Reproducibility	Reference materials, fingerprints, validated assays	Chemometrics/ML, digital libraries	Model transferability and inter-laboratory variation	Require external validation and traceable reference datasets (8,10).

4. CLASSICAL PHARMACOGNOSTIC AUTHENTICATION

Macroscopic and organoleptic examination is an essential first-line defense for identifying crude herbal drugs, since colour, odour, consistency, size, shape, and other characteristics often allow quick identification and detection of obvious adulterants (1,2). These methods are also time- and cost-effective and do not require any sophisticated equipment, making them ideal for use in incoming material control. Microscopic examination can help identify herbal materials by showing special structures such as trichomes, stomata, fibres, vascular bundles, starch grains, and calcium oxalate crystals. It is especially useful for powdered herbs and raw materials that have been damaged during processing. However, microscopy can be difficult to interpret and is less useful for highly processed herbal products because their natural plant structures may no longer be present. The main limitation of traditional pharmacognostic methods is that they provide little information about the chemical composition of the sample. Therefore, these methods should be used along with other chemical and analytical tests to get more accurate information about the quality and identity of herbal medicines. (1,4).

The main limitation of traditional pharmacognostic tests is that they provide only limited information about the chemical contents of herbal medicines and may not be useful for highly processed products. Therefore, these methods should be combined with other chemical and analytical tests to get more complete and reliable information about the identity and quality of the herbal medicine. (1,4,5).

5. PHYSICOCHEMICAL AND PHARMACOPOEIAL STANDARDIZATION

Physicochemical tests such as checking foreign matter, moisture content, ash content, loss on drying, and

extractive values provide basic information about the quality and physical properties of herbal materials. (1). Moisture content is important because excess moisture can increase the risk of microbial growth and spoilage, while ash content gives an idea about the amount of inorganic material present. However, these tests alone cannot identify a particular plant species or give complete information about its chemical composition. Therefore, physicochemical tests should be used together with traditional pharmacognostic and chemical analysis methods. (1,2).

Pharmacopoeial standards can also help in setting quality requirements, but their usefulness depends on whether suitable standards and testing procedures are available for the particular herbal material. (1,3).

6. CHROMATOGRAPHIC FINGERPRINTING AND MARKER-BASED CONTROL

Chromatography is an important method used to test herbal medicines because it helps separate and study the different substances present in complex herbal mixtures. It is useful for comparing herbal samples through fingerprinting and for measuring the amount of specific compounds. HPTLC is mainly useful for quick and simple testing of many samples, while HPLC provides more accurate and detailed quantitative analysis of individual compounds (2,3).

An example of the tremendous potential of chromatography in herbal medicine comes from a survey of commercial herbal medicines that demonstrated the usefulness of HPTLC fingerprinting in revealing non-random variation between different preparations. The same study demonstrated that HPTLC can be used to identify adulteration and counterfeiting by detecting additional peaks that were not present in authentic material (7).

In both HPTLC and HPLC, it is important to detect and identify extra peaks because herbal medicines usually contain many different chemical compounds. These additional peaks can provide useful information about the ingredients present and may also help detect unwanted or unexpected substances. Thus, the mere presence of unexpected peaks may indicate adulteration or substitution, even in the absence of quantitative analysis. On the other hand, reliance on a small number of marker peaks can be misleading due to the complexity of herbal formulations (2,3).

Modern chromatographic research trends indicate that the field is moving away from traditional single-wavelength scanning towards more information-rich, multi-channel fingerprinting techniques. These approaches facilitate a more meaningful separation of peaks and greater insight into the composition of complex mixtures by exploiting the wealth of information in 2-D chromatograms (12).

7. MASS SPECTROMETRY AND MOLECULAR CHEMICAL PROFILING

Hyphenated techniques like GC–MS and LC–MS are applied to different classes of phytoconstituents, with GC being more suitable for the analysis of volatile and semi-volatile constituents, like the essential oils, while LC can be used in the analysis of thermally fragile and polar compounds. The hyphenated techniques can provide structural data useful for elucidating chromatographic features and establishing multiple phytochemical markers (2,3).

The main advantage of advanced hyphenated methods such as LC–MS and others is the possibility to study the complex mixture in more detail than a limited set of markers would allow. The key disadvantages are the high costs of equipment and advanced data analysis, the necessity to have reliable reference data and databases for target compounds, and the complexity of the validation process (2,3).

8. SPECTROSCOPIC TECHNIQUES AND CHEMOMETRICS

FTIR, Raman, UV–vis and NMR are instrumental analytical methods that can be applied to obtain chemical fingerprints of the botanical material with relative simplicity and speed, and sometimes minimal sample preparation (8). Their main advantage in the context of authentication originates from their ability to benefit from the application of multivariate data analysis to explore patterns in chemical data that may not be evident from a simple visual inspection of the spectra.

Chemometrics can be used for exploratory clustering, classification, discrimination and prediction analyses of complex analytical data sets (8). Literature reviews on the application of spectroscopic techniques to herbal authentication mostly focused on geographical origin, agronomic practices, processing and adulteration (8). However, the performance of predictive or discriminative models critically depends on the quality of samples, data pre-treatment, choice of the methods and proper validation (8).

One of the key issues arising in publications is overfitting: a model that performs well on an independent test set or during cross-validation may fail to generalize to an independent, external set with similar but not identical samples. Therefore, external validation, along with open access to the training/test sets, is critical for computational models claiming to have predictive performance (8,10,11).

9. DNA-BASED AUTHENTICATION

DNA barcoding is a technique that utilizes the sequence variation between particular taxonomically informative genomic regions as a type of phylogenetic marker that can be used to identify species. Specific regions include *rbcL*, *matK*, *ITS/ITS2* and *trnH-psbA*, which show differential degrees of informativeness and applicability to different groups of organisms (5,6,9).

The main advantage of DNA-based authentication is that it can be used even in cases where the morphology of the sample is insufficient for identification at the species level. However, DNA barcoding is not always successful for processed products due to the decreased quantity and quality of DNA and the potential inability to distinguish between closely related species with available primers (5).

Mini-barcoding, metabarcoding, high-resolution melting analysis and other techniques are currently under investigation for the use in authentication of herbal medicines, which has the potential to increase the performance for processed materials, but each requires proper validation, appropriate reference materials and expert knowledge for interpretation (6,9).

Most importantly, DNA-based authentication methods should not be considered as a replacement for chemical analysis. While DNA analysis can confirm the presence of a species, it does not provide information on the chemical profile of the sample and its conformity to the requirements. This is especially important for processed materials: even if a DNA barcode confirms the presence of a species, it does not indicate whether other, potentially harmful, constituents are present in sufficient quantities.

10. METABOLOMICS: FROM MARKER TESTING TO HOLISTIC CHEMICAL PROFILING

Metabolomics is a broad term that refers to studies of metabolites and metabolic processes. It encompasses a wide range of analytical techniques, among which are NMR, LC–MS and GC–MS, which have been applied to herbal materials to reveal characteristic patterns or markers indicative of species, geographical origin, processing or quality (13). Metabolomics data can be subjected to multivariate statistical analysis to identify major variables that can be used as chemical markers or fingerprints (13).

The strength of this approach lies in the fact that it allows the characterization of herbal materials beyond a few selected markers, potentially revealing important information on the quality or authenticity of a sample. The key limitation of such approach is that metabolomics data is complex and high-dimensional and requires significant effort to analyze and interpret (13). Additionally, such analyses are highly dependent on the choice of analytical methods and sample preparation and have limited opportunities for rapid, routine screening (13).

For this reason, metabolomics data analysis is better suited for discovery or discrimination purposes rather than routine quality control. For routine applications, it may be more practical to use targeted biochemical assays to identify specific markers, with metabolomics used to characterize the fingerprint and identify patterns for discovery or discrimination purposes (2,13).

11. ARTIFICIAL INTELLIGENCE, MACHINE LEARNING AND HYPERSPECTRAL IMAGING

Machine learning algorithms can be used for a variety of analytical tasks, including the identification of patterns and features characteristic of species or geographical origin. The recent literature highlights the work on combining hyperspectral imaging data with mass spectrometry and NMR data and utilizing machine learning algorithms for pattern recognition and interpretation in the context of quality assessment of herbal medicines (10).

Hyperspectral imaging is another emerging technology that has been explored for the quality assessment of herbal medicines using machine learning algorithms. Its major advantage is the possibility to rapidly assess botanical materials in a non-destructive manner, with spatial resolution, but the major limitations include the complexity of data partitioning, pre-treatment and dimensionality reduction, as well as model selection and performance assessment (10).

The current challenge for this review is the fact that the state of the art in the application of analytical technologies for the identification of patterns has already been reviewed and published in 2026 (10). As such, the review considers the key research gap to be the practical application of these methods: how can such models be externally validated, used in different laboratories with different equipment, validated against reference materials and interpreted by regulators, and how can the changes in the model be tracked during changing botanical and manufacturing conditions (10,11)?

12. SAFETY AND CONTAMINANT CONTROL

It is important to note that chemical authenticity and botanical identity are not the same as safety: herbal medicines may require additional safety evaluations depending on the level of contaminants, such as bacteria, mycotoxins, pesticide residues, toxic elements or others that may have accumulated depending on the growing conditions, processing and other factors (1).

The aforementioned differences are relevant to the discussion on the development of the integrated framework because it is entirely possible that a sample passes the botanical and chemical authentication tests but fails the safety evaluation, or that a sample has sufficient characteristics to pass a limited set of chemical tests for authenticity but fails the botanical identification. Therefore, it is important to distinguish between the two types of analyses and consider safety testing as an independent procedure. At the same time, the selection of the appropriate tests must be driven by the risk assessment and must follow the principles of analytical validation(1).

13. REGULATORY AND TRANSLATIONAL CONSIDERATIONS

The quality control guidelines issued by the World Health Organization and pharmacopoeias of various countries serve as the basis for identity, purity and other analytical tests for herbal medicines, while good agricultural and collection practices, and good manufacturing practices, set the standards for processing and manufacturing (1,14). The current challenge is the fact that regulatory requirements and available monographs significantly vary between jurisdictions and analytical advances are not always directly applicable to the routine requirements for quality control.

For an analytical method to be used in routine quality control, it must be sufficiently simple, robust and validated for these purposes. Computational methods have additional requirements, including the necessity to store large volumes of data, have consistent and auditable data analysis pipelines, and ensure traceability and repeatability of results (3,10).

The regulatory strategy that appears to be the most efficient for the time being is the concept of tiered testing: simple tests for the majority of materials, more involved analytical methods for specific quality requirements, and advanced methods such as mentioned above for the materials that require extensive profiling and are difficult to characterize otherwise (1,2,10).

14. PROPOSED INTEGRATED QUALITY-ASSURANCE WORKFLOW

The framework suggested in this review starts with risk assessment and material characterization, followed by the first-level testing of botanical identity and physicochemical properties. If required, the molecular authentication can be applied; if the initial chemical tests suggest that adulteration or inconsistency is possible, the reference comparisons with authenticated materials can be undertaken; and in the case of complex, high-value or at-risk materials, the metabolic profiling can be conducted (1–6,8,9).

The final decision on the quality should be made on the basis of a combination of available pieces of information rather than a single analytical method or test, with inconsistent results prompting further inquiry rather than being averaged out into a positive result. This approach is in line with the general principle that different analytical methods measure different attributes of a complex product such as botanical materials (2,5,9).

Table 2. Proposed decision-oriented workflow for herbal quality assurance

Stage	Primary question	Preferred evidence	Decision trigger
1. Risk assessment	What is the product and matrix?	Raw material/product history, intended use, processing level	High-risk or highly processed material → advanced testing
2. Identity	Is the declared botanical material present?	Macroscopy/microscopy ± DNA authentication	Discordant identity evidence → investigation
3. Purity	Is substitution/admixture evident?	Microscopy, HPTLC/HPLC, DNA, spectroscopy	Unexpected zones/sequences or classification anomaly
4. Chemical consistency	Does the chemical profile conform?	Fingerprint + marker assay ± LC-MS/NMR	Fingerprint or marker failure

3. Purity	Is substitution/admixture evident?	Microscopy, HPTLC/HPLC, DNA, spectroscopy	Unexpected zones/sequences or classification anomaly
4. Chemical consistency	Does the chemical profile conform?	Fingerprint + marker assay $\pm$ LC-MS/NMR	Fingerprint or marker failure
5. Safety	Are relevant contaminants controlled?	Microbiology, mycotoxin, pesticide and elemental methods	Any result exceeding specification
6. Data integration	Do evidence streams agree?	Chemometrics/ML where validated	Model uncertainty or discordance $\rightarrow$ confirmatory test
7. Release/traceability	Is the quality decision defensible?	Validated specifications, reference materials, records	All critical attributes conform

15. RESEARCH GAPS AND FUTURE DIRECTIONS

A significant limitation of many studies concerned with advanced authentication approaches is a lack of representative reference sets of materials as an essential basis for comparative identification or classification. For DNA-based authentication, the availability of extensive databases of sequence variants is a prerequisite for reliable identification. Similarly, for chemical and spectroscopic analysis, reference sets of materials are required that represent the natural intraspecific or intravarietal variability. An inadequate reference set, therefore, may lead to apparently successful classifications (5,8,10).

Another limitation of many studies concerned with analytical authenticity control is the lack of external validation, robustness against analytical or instrumental variability, and uncertainty analysis, as well as false discovery and false-negative rates with independent test sets. This is especially true for machine learning studies, in which the performance of different algorithms often strongly depends on the choice of training and test sets, or in the case of information leakage between these sets (10,11).

A further limitation is the lack of consideration of the transition from novel analytical discoveries to routine practice: Even if metabolomics or machine learning enable new insights into the biological underpinnings of the taxonomic or compositional variability, implementing such approaches into regulatory practice requires additional translational research (10,13).

Therefore, future studies should consider the development of compatible reference libraries, standardized sample preparation, and multisource validation, as well as the consideration of different analytical data types and the derivation of appropriate decision thresholds for risk assessment. More advanced screening assays, for example, rapid and portable spectroscopy, may be applied to supply chains or the field, while laboratory-based confirmation methods would still be used for questionable findings (6,9,11).

16. NOVELTY AND CONTRIBUTION OF THE PRESENT REVIEW

The novelty of the present work was not related to the specific analytical technology per se, since recent literature already described DNA-based orthogonal authentication approaches, analytical technologies combined with chemometrics, and the use of machine learning in combination with analytical measurements for authentication purposes (8–10). Therefore, the contribution of the present review was related to the general concept of decision-oriented quality assessment.

To the best of our knowledge, this review proposes a decision-oriented framework of quality assessment strategies for herbal medicines with an emphasis on the complementary nature of different analytical evidence types for quality assessment. In particular, the distinction between different quality-related hypotheses related to the botanical identity, purity/adulteration, chemical consistency, safety, and reproducibility was made. The critical evaluation of the applicability of different analytical and computational methods for each of these hypotheses was undertaken, highlighting the general principles for selecting the relevant analytical technologies.

Furthermore, the critical analysis of the necessity of the establishment of reference materials and external validation, the importance of reproducibility, and interpretability of findings was performed. This aspect is especially relevant for the consideration of the transition from promising analytical developments to routine regulatory practice. Such an approach, therefore, may serve as a conceptual framework for making evidence-based decisions on analytical methods for herbal medicines, distinguishing between different levels of analytical and computational evidence (1–3,8–10).

17. CONCLUSION

Quality assessment of herbal medicines requires multiple lines of evidence because of the complexity of the quality-related hypotheses related to botanical identity, chemical consistency, and safety. Whereas classical pharmacognostic methods remain critical for first-line testing, chromatographic, spectroscopic, molecular, and computational approaches allow addressing more complex questions of adulteration, chemical inconsistency, and safety.

The decision-oriented framework provided in this article may serve as the basis for selecting the most relevant strategies for quality assessment of herbal medicines, depending on the product category and the analytical questions. This framework emphasizes the need to consider both pharmacognostic principles (such as the selection of relevant botanical species and chemotypes) and advanced computational approaches, such as machine learning, for the analysis of complex data. For example, DNA barcoding can provide valuable molecular evidence for botanical authentication, particularly when morphological identification is difficult, although its applicability depends on DNA quality, barcode resolution and the availability of reliable reference sequences.(5,8,10).

Thus, the future trends in the quality assessment of herbal medicines are related to the establishment of integrated, orthogonal, and interoperable evidence rather than the use of individual analytical methods for quality assessment. Emphasis should be placed on the establishment of validation, reference data, and standardization of procedures at the analytical, computational, and regulatory levels rather than the mere selection of individual components for analytical and computational technologies. This approach would enable both the use of pharmacognostic heritage and the opportunities provided by innovative analytical and computational methods, as well as the ability to make accurate and defensible quality assessments of herbal medicines(1,3,10).

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