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Botanical Authentication Under Adulteration Pressure: A Systematic Review of DNA, Chromatographic, Spectroscopic, and Chemometric Evidence for Medicinal-Plant Identity Control

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ABSTRACT

Medicinal-plant authentication is increasingly challenged by substitution, undeclared admixture, processing-related signal loss, compositional variability, and deliberate manipulation of routine quality-control markers. DNA-based, chromatographic, spectroscopic, and chemometric approaches are often discussed as competing solutions, although they measure different properties and do not support identical identity claims. A systematic review of peer-reviewed Q1 journal literature published from 2017 to 2023 was conducted using botanical-authentication, adulteration, DNA, chromatography, spectroscopy, mass-spectrometry, fingerprinting, and chemometric search concepts. Eligible studies and substantive methodological reviews were required to support a defined authentication claim, analytical limitation, validation issue, or boundary condition. Evidence was extracted by material state, identity endpoint, analytical modality, comparator, validation design, and uncertainty source, then synthesized narratively because outcome definitions and performance metrics were heterogeneous. Thirty-four articles were retained from a verified candidate pool of 50. DNA methods principally supported taxonomic presence or identity, whereas chromatographic, NMR, mass-spectrometric, and vibrational methods supported chemical-profile or constituent evidence. Chemometrics enabled discrimination within defined reference spaces but introduced dependence on preprocessing, class definition, and validation. Processing, incomplete reference libraries, unseen adulterants, and deliberate marker manipulation repeatedly constrained transferability. No single analytical family provides context-independent botanical authentication. This review proposes that method suitability should be interpreted through alignment among the claimed identity attribute, matrix state, adulteration mechanism, surviving analytical signal, authenticated comparator, and validation design. Orthogonal evidence is particularly informative when these layers can decouple.

Keywords: Botanical authentication, Herbal adulteration, DNA barcoding, Chemical fingerprinting, Spectroscopy, Chemometrics

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Introduction

Medicinal-plant identity control operates in a market where substitution, mislabeling, undeclared ingredients, and deliberate attempts to satisfy or defeat routine tests can coexist. Commercial-product studies and forensic syntheses show that authentication failure is not confined to one botanical group or analytical modality, and an adulterated material may still appear compliant when an assay interrogates only a narrow marker or evidence layer [1–3]. This matters because “identity” is not a single observable property. DNA-based authentication can establish taxonomic presence or sequence-level correspondence when recoverable genetic material and adequate references

are available, but it does not by itself establish expected chemical composition or the abundance of characteristic constituents [4].

Chemical fingerprints and targeted constituent measurements interrogate a different evidential layer. Their interpretation, however, can be affected by provenance, harvest, processing, extraction, storage, formulation, and analytical conditions. Chemometric workflows can extract discriminatory structure from complex profiles, yet conclusions depend on preprocessing, reference-class construction, feature selection, leakage control, and validation strategy [5]. Consequently, a high classification rate in one study cannot be treated as an intrinsic or transferable property of a technology. Reviews spanning contemporary analytical platforms likewise show that authentication performance is conditional on measurement system, sample matrix, comparator, and modelling design [6].

DNA technologies have expanded beyond conventional single-locus barcoding to metabarcoding, high-resolution melting, mini-barcodes, and plastome-informed approaches, but reference completeness and DNA degradation remain consequential constraints [7]. The resulting literature therefore presents an evidence-integration problem rather than a simple technology-ranking problem. A method may be highly informative for species substitution but weak in a highly processed extract; another may characterize chemical conformity while remaining unable to distinguish an authentic but chemically variable material from a taxonomic substitute sharing major metabolites.

This review treats botanical authentication under adulteration pressure as a layered analytical-inference problem in which the meaning of a positive or negative result depends on what attribute was measured, what material state was tested, what adulteration mechanism is plausible, and what validation supports the inference. Its purpose is not to designate a universal best method, but to determine where different evidence families are informative, where their interpretations fail, and when their combination materially changes confidence in medicinal-plant identity control.

Review objectives

The primary objective was to systematically evaluate how DNA-based, chromatographic, spectroscopic, mass-spectrometric, and chemometric evidence has been used to authenticate medicinal plants and commercial herbal products under realistic or experimentally defined adulteration conditions. The review distinguishes four questions that are frequently merged in practice: whether the declared taxon is present, whether an undeclared taxon is present, whether the material exhibits an expected chemical profile, and whether a multivariate model assigns the sample to a predefined class. DNA-based methods are especially relevant to the first two questions [4], whereas chemical and chemometric methods address compositional and classification evidence without automatically proving species identity [5].

A second objective was to compare analytical performance without treating heterogeneous metrics as commensurable. Rather than ranking technologies by isolated accuracy values, the review considers the identity endpoint, physical state of the test material, adulterant universe, analytical reference, validation strategy, and transfer conditions that give a reported result meaning. Contemporary authentication reviews demonstrate why this contextualization is necessary: analytical platforms differ in what they measure and in the sources of variability carried into the final assignment [6].

The third objective was to map method suitability against adulteration mechanisms. These include whole-species substitution, undeclared admixture, processed or degraded material, compositional dilution, chemically similar substitution, and deliberate manipulation of expected analytical markers. Chemical-authentication literature demonstrates the vulnerability of narrow constituent testing [2], while forensic evidence shows that some adulteration strategies are specifically designed to exploit predictable analytical routines [3].

On this basis, the review develops a proposed evidence-bounded synthesis in which authentication confidence depends on alignment among the claimed identity attribute, matrix state, adulteration mechanism, surviving analytical signal, authenticated comparator, and validation design. This synthesis is an organizing framework derived from the reviewed evidence, not a prospectively validated decision rule. It is intended to clarify when orthogonal methods answer genuinely different questions and when apparent analytical agreement can still rest on a shared evidential weakness.

Materials and Methods

The review was conducted and reported in alignment with PRISMA 2020 principles [8]. Searches combined medicinal-plant and herbal-product terminology with authentication, adulteration, substitution, contamination, DNA barcoding, metabarcoding, chromatography, mass spectrometry, NMR, infrared, Raman, fingerprinting, chemometrics, classification, and validation concepts. Eligible articles were peer-reviewed journal papers published from 2017 through 2023, had a verifiable DOI, satisfied the predefined Q1 journal criterion in a relevant publication-year category, and supported a specific authentication claim, methodological decision, limitation, or boundary condition. Conference papers, preprints, non-journal material, duplicate versions, out-of-range publications, and adjacent studies without a direct identity-control role were excluded.

Extraction captured material state, botanical or ingredient target, adulteration problem, analytical modality and representation, comparator, reference provenance, validation design, principal finding, uncertainty source, and transferability boundary. A structured analytical-evidence appraisal considered authenticated reference materials, matrix relevance, comparator adequacy, analytical replication, chemometric leakage control, independence of validation, and external or interlaboratory confirmation. This appraisal calibrated interpretation but was not treated as a validated risk-of-bias instrument.

Because tasks, matrices, metrics, and validation designs were heterogeneous, synthesis was narrative rather than meta-analytic. No prospective protocol registration was available for reporting. The verified selection ledger preserved a DOI-deduplicated candidate pool of 50 articles, with 34 retained and 16 excluded after final eligibility and evidence-fit assessment. Database-specific raw yields and stage-resolved full-text counts were not reconstructed because they were not preserved in the auditable search record.

Figure 1 reports the verified selection transitions available from that record without estimating unavailable identification-stage

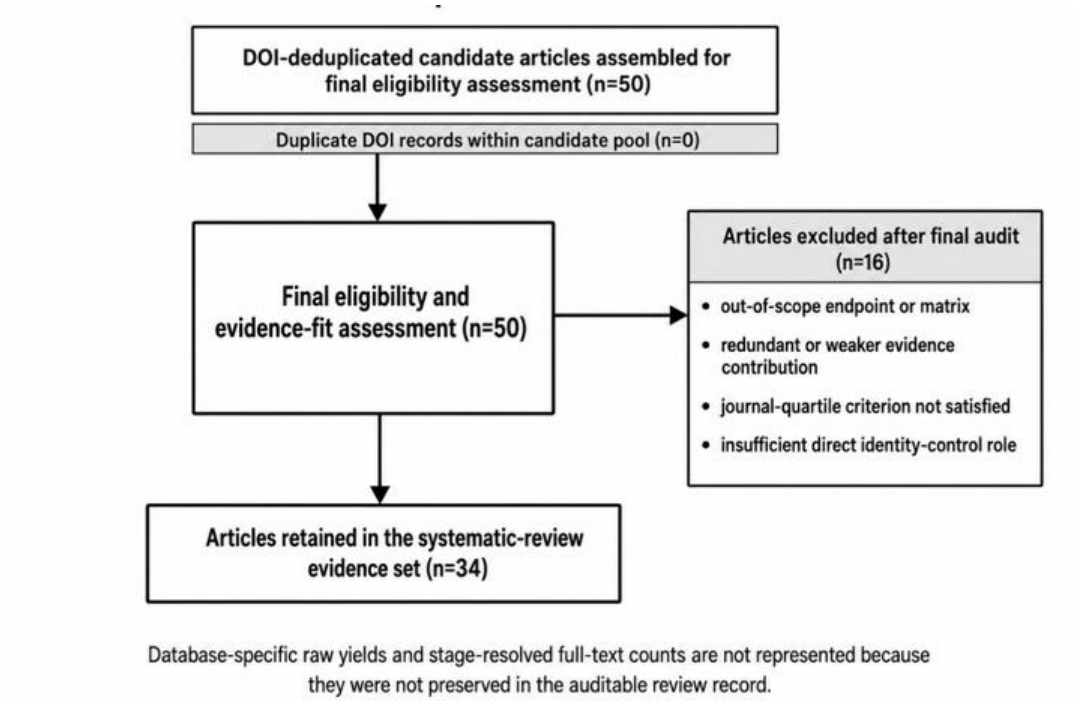

Figure 1. Verified candidate-level evidence-selection flow for the medicinal-plant authentication review

The evidence dimensions used to structure extraction and interpretation are summarized in **Table 1**.

Table 1. Evidence characteristics governing interpretation of medicinal-plant authentication studies across analytical modalities

Evidence characteristic	Extracted question	Interpretive consequence
Material state	Raw, powdered, extract, oil, tea, or finished formulation?	Determines whether DNA, metabolites, or spectral patterns remain measurable

Identity endpoint	Taxon presence, undeclared taxon, chemical conformity, or class assignment?	Prevents non-equivalent claims from being compared as one outcome
Reference basis	Authenticated voucher, curated sequence, chemical standard, or training class?	Defines the comparator against which "authentic" is assigned
Adulteration context	Substitution, admixture, dilution, processing, or marker manipulation?	Changes which analytical signal is informative
Validation design	Internal, cross-validated, external, or interlaboratory?	Governs confidence in transfer beyond the fitted dataset
Uncertainty source	Degradation, amplification bias, matrix variation, batch effects, or domain shift?	Constrains negative results and model-based classifications

Study selection and evidence characteristics

The retained evidence combined commercial-product investigations, targeted method-development experiments, orthogonal analytical comparisons, reference-library studies, and methodological syntheses. Early direct comparisons demonstrated why modality must be interpreted explicitly. In commercial *Hypericum* products, DNA metabarcoding, TLC, and HPLC-MS interrogated overlapping but non-identical evidence about labelled composition [9]. An *Echinacea* study pairing DNA metabarcoding with HPTLC similarly showed that taxonomic and chemical observations should not be treated as interchangeable authentication endpoints [10].

Complex formulations intensified this distinction. DNA metabarcoding of Ayurvedic products identified discrepancies between labelled and detected botanical components, while interpretation remained dependent on recoverable DNA and reference coverage [11]. Dual-marker authentication of herbal teas further showed that marker choice influences detectability and that read abundance should not be equated directly with ingredient quantity [12]. Multi-barcode sequencing extended taxonomic analysis into processed traditional Chinese medicine preparations [13]. Conversely, construction of a Thai pharmacopoeial barcode library illustrated that sequence-based assignment is only as defensible as the authenticated reference space supporting it [14].

The evidence also contained targeted and orthogonal designs addressing narrower analytical questions. High-resolution melting analysis was applied to *Hippophae* species discrimination and commercial-product authentication within a defined taxonomic setting [15]. DNA sequencing combined with toxicological screening demonstrated that botanical identity and hazardous undeclared content can require complementary analytical endpoints [16]. Plastome sequencing improved discrimination among closely related *Polygonatum* materials where shorter loci may be insufficient [17]. In *Mucuna*, HPLC and DNA barcoding jointly supported product identification and detection of prohibited material [18]. A milk-thistle study combining DNA metabarcoding with UHPLC-QTOF-MS was particularly informative because chemical evidence could remain interpretable where DNA detection was limited, showing that cross-method discordance can arise from the evidence layer rather than simple analytical failure [19].

Accordingly, the retained evidence was compared by material state, identity endpoint, reference basis, and validation design rather than technology name alone. Raw botanicals, degraded processed products, extracts, and multicomponent finished formulations were not treated as analytically equivalent matrices.

Authentication technologies represented in the evidence

DNA-based approaches supplied the most direct evidence for taxonomic presence, substitution, or undeclared botanical components when amplifiable DNA and suitable references were available. In commercial *Veronica* products, DNA metabarcoding combined with HPLC-MS identified substitution by a related species [20]. *Garcinia* authentication showed a complementary division of analytical labor: DNA addressed species identity, whereas NMR characterized constituent composition [21]. For processed *Senna*, a plastome-derived mini-barcode improved identification where conventional barcode regions were vulnerable to DNA degradation [22]. These studies support conditional rather than universal claims for DNA sufficiency.

Chemical and spectroscopic methods interrogated composition through different signal representations. Multi-source and non-destructive approaches broaden the information available for rapid herbal authentication, although their usefulness remains dependent on matrix and validation context [23]. A horizon scan of DNA-based quality control likewise emphasized that technological advances do not remove limitations arising from reference coverage, processing, and implementation [24]. Fingerprint analysis captures multicomponent chemical patterns rather than isolated marker compounds, but defensible comparison requires standardized acquisition and reference

procedures [25]. Infrared and Raman approaches provide rapid profile-based discrimination while remaining susceptible to calibration robustness and transfer limitations [26].

Richer chemical representations can also be coupled to multivariate classification. Heating online extraction electrospray ionization mass spectrometry generated rapid discriminating profiles across a defined herbal set [27]. NMR-based chemical barcoding transformed reproducible multicomponent spectra into profile-level identity information within a specified reference space [28]. Direct comparison of UV, FTIR, and ^1H NMR in turmeric showed that different spectroscopic modalities do not necessarily encode equivalent discriminatory information [29]. Controlled saffron-adulteration experiments demonstrated that diffuse-reflectance infrared spectroscopy combined with chemometrics could detect defined plant-derived adulterants, while also illustrating the less demanding conditions of a known-adulterant experiment relative to open-world commercial fraud [30].

Chemometrics therefore functions as a cross-cutting interpretive layer rather than an independent source of botanical identity evidence. It converts chromatographic, mass-spectrometric, NMR, or vibrational measurements into class separation or prediction, but the resulting inference remains bounded by the measurement process, reference classes, adulterant universe, and validation design from which the model was learned.

These differences are synthesized in **Table 2** according to the signal measured, the identity claim it can most directly support, and the principal conditions governing interpretation.

Table 2. Analytical evidence families and the identity claims they support in medicinal-plant authentication

Evidence family	Primary signal	Most direct authentication use	Principal vulnerability	Validation priority
DNA barcoding / metabarcoding	Recoverable taxonomic sequence	Declared or undeclared taxon detection	DNA degradation, amplification bias, incomplete references	Authenticated sequence library and matrix-relevant recovery
Chromatography / MS	Multicomponent chemical profile	Chemical conformity and profile deviation	Biological/process variability, narrow reference space	Standardized acquisition and external reference materials
NMR profiling	Metabolite pattern and constituent signals	Orthogonal chemical identity and composition	Concentration and formulation effects	Replicate reproducibility and broader authentic-material libraries
IR / Raman spectroscopy	Bulk vibrational signature	Rapid screening and defined-adulterant discrimination	Instrument, batch, particle-size, and domain effects	Locked preprocessing and external-domain testing
Chemometrics	Statistical structure in measured features	Classification within predefined classes	Overfitting, leakage, class imbalance, unseen adulterants	Independent validation with prespecified classes
Orthogonal multimodal testing	Convergent or discordant signals	Resolving taxonomic–chemical ambiguity	Added complexity and discordant-result interpretation	Predefined logic for agreement and disagreement

Comparative analytical performance

Analytical performance was not meaningfully comparable as a single technology-level quantity because the reviewed studies differed in endpoint, adulterant universe, matrix, reference classes, preprocessing, and validation strategy. Raman spectroscopy coupled to chemometric classification differentiated authentic and adulterated agarwood essential oils within its defined analytical domain [31]. This demonstrates useful discrimination under a specified reference space, but it does not establish equivalent transfer to powders, extracts, multicomponent preparations, or previously unseen adulterants. Direct commercial-product studies likewise show that DNA-based and chemical-profile methods can provide complementary but non-equivalent authentication evidence [9, 10, 19].

Validation design was consequently treated as part of the authentication claim rather than as a secondary statistical detail. In *Rhizoma Alismatis*, metabolomic profiling was combined with quantitative marker assessment, support-vector classification, and explicit cross-validation, strengthening the evidential connection between chemical patterns and predefined botanical-origin classes [32]. Nevertheless, the resulting model remains conditioned by the represented origins and reference materials. FT-NIR analysis of *Ziziphi Spinosae Semen* similarly enabled

rapid discrimination from investigated adulterants, while prediction beyond the represented sample and adulterant domain remains uncertain [33]. HPLC-UV coupled to chemometrics also supported classification of herbs and aromatic materials within a defined sample universe [34].

These studies indicate that headline classification metrics cannot be detached from experimental design. Internal separation may demonstrate that a signal exists, whereas external prediction asks whether that signal survives new batches, instruments, provenances, processing states, or adulterants. Spectral and chromatographic methods can therefore be highly efficient screening tools without becoming independent taxonomic proof. Conversely, DNA can resolve taxonomic substitution without establishing expected concentrations of chemically active or characteristic constituents.

The review therefore interprets comparative performance through four linked questions: what identity attribute is being claimed; whether the relevant signal survives in the tested matrix; whether authentic and adulterated reference classes adequately represent the intended decision space; and whether validation tests transfer rather than merely refit that space. On this basis, analytical superiority is conditional rather than absolute.

Adulteration types and method suitability

Adulteration mechanism strongly modifies method suitability. Whole-species substitution is particularly amenable to DNA-based authentication when discriminatory sequence information survives processing. Targeted high-resolution melting successfully distinguished tested *Hippophae* species and commercial materials within a defined assay [15]. DNA and HPLC together supported discrimination of *Mucuna* materials and prohibited products [18]. DNA metabarcoding with HPLC-MS identified replacement of declared *Veronica officinalis* by *V. chamaedrys* in commercial material [20]. These examples support taxonomic testing when the primary question is whether the declared botanical identity is present.

Undeclared admixture and multicomponent formulations are more difficult. Metabarcoding can reveal additional taxa in complex herbal products, but primer bias, DNA degradation, and incomplete reference libraries complicate interpretation of non-detection [11]. Orthogonal toxicological testing can additionally detect undeclared hazards that taxonomic sequencing alone does not characterize [16]. In finished milk-thistle formulations, differences between DNA and metabolomic findings showed that failure to recover DNA need not imply absence of chemically recognizable botanical material [19].

Processing further changes signal availability. Multi-barcode sequencing can recover ingredients from processed traditional preparations when amplifiable DNA persists [13], whereas plastome-derived mini-barcodes can improve identification of degraded material [22]. DNA-method assessments nevertheless retain processing and reference completeness as fundamental constraints [24]. At the chemical level, narrow marker conformity may also be misleading when environmental variability or deliberate marker manipulation produces apparently acceptable results [2]. Forensic analyses show that sophisticated adulteration can exploit predictable single-marker tests [3], while broad NMR fingerprints provide richer compositional evidence but remain dependent on authentic reference diversity [28].

The evidence therefore supports no fixed one-method-to-one-adulterant mapping. Instead, this review proposes that suitability depends jointly on adulteration mechanism, matrix state, target identity attribute, signal persistence, and validation scope. **Figure 2** visualizes this proposed context-conditioned interpretation and its principal uncertainty boundaries.

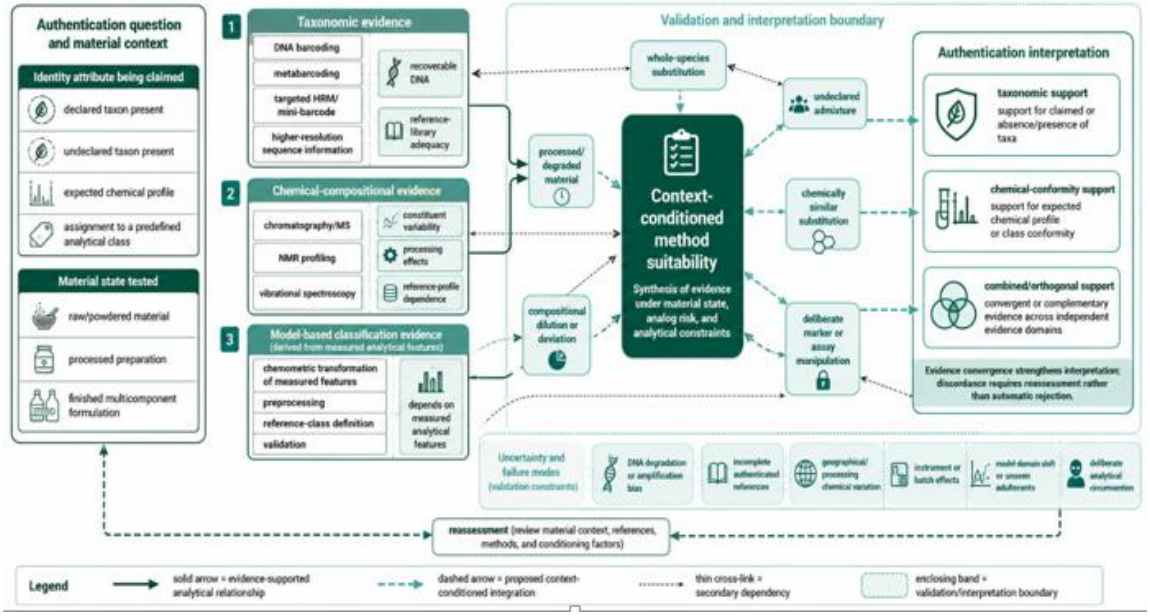

Figure 2. Context-conditioned integration of taxonomic, chemical, and model-based evidence for botanical authentication under adulteration pressure

Results and Discussion

The reviewed evidence indicates that botanical authentication should be understood as an inference about a specified identity attribute rather than as the output of a particular instrument. DNA evidence can directly support taxonomic presence when recoverable sequence and adequate references exist, whereas chemical evidence can establish conformity with expected compositional patterns without independently proving botanical taxonomy. Studies applying both evidence classes to the same products demonstrate why these distinctions matter: agreement can strengthen interpretation, but disagreement can arise because the analytical methods measure different surviving properties rather than because one method necessarily failed.

Accordingly, method selection should be fit-for-purpose and validation-sensitive rather than governed by a universal technology hierarchy [5, 6, 24]. The principal synthesis proposed here is an alignment model: the evidential strength of an authentication result depends on whether the identity claim, material state, adulteration mechanism, analytical signal, reference comparator, and validation design are mutually compatible. This is not a prospectively validated scoring system. It is a reconstruction of conditions repeatedly exposed by the reviewed literature.

The implications are especially important under adversarial conditions. Reliance on a single characteristic compound may be vulnerable when adulteration preserves, supplements, or mimics that marker [3]. Broader fingerprints can reduce dependence on individual constituents, but their interpretation still requires standardized analytical conditions and representative authentic materials [25]. Spectroscopic classification similarly gains practical value from speed and low sample preparation, while robustness beyond the calibration domain remains an evidential requirement [26]. Cross-validated metabolomic classification illustrates how stronger model design can improve confidence without eliminating dependence on the represented botanical domain [32].

Orthogonal testing is therefore most valuable when methods contribute genuinely different information. It should not be equated with simply repeating several correlated chemical assays. The review instead supports a distinction between convergence across evidential layers and redundancy within one layer. **Table 3** summarizes this synthesis together with the main conditions that presently prevent conversion into a universal authentication standard.

Table 3. Evidence synthesis, unresolved gaps, and proposed implications for botanical identity control

Synthesis issue	Evidence-supported interpretation	Unresolved gap	Proposed implication
Taxonomic versus chemical identity	DNA and chemical profiles answer different identity questions	No universal rule for resolving discordance	Treat evidence layers explicitly rather than interchangeably

Processed materials	Processing can reduce DNA recovery and alter composition	Matrix-specific recovery is inconsistently validated	Match method to signal expected to survive processing
Reference quality	Authentication depends on authenticated sequences or chemical comparators	Reference breadth and provenance remain uneven	Make reference adequacy part of the authentication claim
Chemometric classification	Models can discriminate represented classes	External transfer and unseen adulterants remain uncertain	Require validation outside the fitted reference domain
Deliberate adulteration	Narrow markers can be circumvented	Adversarial challenge sets are uncommon	Use broader or orthogonal evidence where deception is plausible
Multimodal evidence	Independent evidence layers can strengthen interpretation	Rules for discordant results are underdeveloped	Predefine reassessment rather than treating disagreement as automatic failure

Methodological and validation gaps

Reference infrastructure remains a limiting determinant of authentication quality. DNA assignment depends on authenticated and sufficiently comprehensive sequence libraries, while close taxa may require higher-resolution genomic information when standard loci are inadequate [7]. Reference-library construction itself therefore constitutes part of analytical validation rather than a background bioinformatics task [14]. Plastome-scale approaches can increase discrimination in difficult taxonomic groups, but their broader necessity and cost-effectiveness remain context dependent [17]. Recent assessments continue to identify reference coverage and implementation consistency as unresolved limitations [24].

Processed products present a second gap. Marker performance can differ substantially in mixtures, and amplification biases restrict quantitative interpretation of metabarcoding signals [12]. Multi-barcode approaches increase recovery opportunities in processed preparations but cannot detect DNA that has been irreversibly degraded or inadequately extracted [13]. Discordant DNA and chemical findings in finished products further demonstrate why negative DNA evidence requires qualification [19]. Mini-barcode strategies mitigate some degradation problems but do not eliminate assay-specific bias [22].

Chemometric validation is comparably uneven. Model performance is conditioned by preprocessing, feature selection, class construction, and separation between training and validation data [5]. Spectroscopic reviews identify robustness across instruments and real-world production conditions as continuing needs [26]. Stronger studies incorporate explicit cross-validation or independent testing, yet botanical provenance, new adulterants, and laboratory transfer can still generate domain shift [32]. Future work should prioritize blinded multisite comparisons, locked analytical pipelines, authenticated challenge materials, deliberately difficult adulterants, and predefined interpretation of cross-method discordance.

Limitations

The principal limitation of this review is heterogeneity. Included evidence spans raw botanicals, powders, extracts, essential oils, teas, finished formulations, targeted adulteration experiments, and commercial-market surveys. Authentication endpoints likewise range from taxonomic detection to chemical-profile conformity and supervised classification. These differences preclude meaningful quantitative pooling and prevent direct ranking of nominal performance values across studies [5]. Cross-platform reviews similarly indicate that technologies operate under different analytical assumptions and validation conditions [6].

Commercial-product studies also cannot establish a universal prevalence of adulteration. Sampling frames, countries, product categories, taxonomic targets, analytical methods, and definitions of nonconformity differ substantially [1]. Findings from complex Ayurvedic formulations illustrate both the power and interpretive difficulty of broad DNA screening in multicomponent products [11], while toxicological and DNA testing of regulated supplements addresses a different market and risk structure [16]. Such evidence is informative about possible failure modes but should not be extrapolated as a global rate.

The review was additionally restricted by its predefined publication-period, journal-quality, and DOI eligibility criteria, which increase consistency but exclude potentially relevant evidence outside that frame. The auditable search record preserved a verified candidate pool and final eligibility decisions but not database-specific raw yields or stage-resolved retrieval counts. Consequently, **Figure 1** reports only verified selection transitions rather

than reconstructing unavailable PRISMA counts. Finally, the context-conditioned suitability architecture proposed here is derived from heterogeneous published evidence and requires prospective testing before it can function as a decision instrument.

Conclusion

Medicinal-plant authentication under adulteration pressure cannot be reduced to a contest between DNA, chromatography, spectroscopy, and chemometrics. These approaches interrogate different properties, operate under different matrix constraints, and support different levels of identity inference. Their apparent agreement or disagreement therefore has meaning only when the claimed botanical attribute, surviving analytical signal, authenticated comparator, and validation design are made explicit.

This systematic review proposes a context-conditioned interpretation in which method suitability depends jointly on the identity question, material state, plausible adulteration mechanism, reference infrastructure, and transferability of the analytical model. DNA is especially informative for taxonomic presence when recoverable genetic material and suitable references exist; chemical profiling is central to compositional conformity; spectroscopy offers rapid pattern-based screening; and chemometrics transforms such measurements into class assignments whose validity cannot exceed the domain used to develop and test them.

The practical implication is not that every botanical product requires maximal multimodal testing. Rather, orthogonal evidence becomes most valuable where processing, complex formulation, chemically similar substitution, unexpected adulterants, or deliberate assay manipulation can decouple taxonomic and chemical signals. Future validation should therefore emphasize authenticated challenge materials, external and interlaboratory testing, explicit negative-result interpretation, and predefined handling of discordant evidence. Until such validation is broader, the proposed architecture should be treated as an evidence-organizing framework rather than a universal authentication standard.

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