

Stage-Specific Authentication of Chinese Medicinal Plant Materials from a Supply-Chain Perspective

-- Integrating Morphological, Chemical, and DNA Evidence

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Abstract: After harvest, Chinese medicinal plant materials pass through collection, primary processing, trade, decoction-piece preparation, and finished-product manufacture. Their visible form changes at each step. So does the evidence used for authentication. A single test is therefore unlikely to cover the whole chain with the same reliability. This review considers authentication from a supply-chain perspective, with particular attention to multiple botanical sources and taxonomic ambiguity. It then compares morphological, microscopic, chemical, spectroscopic, and DNA-based approaches, including newer targeted and high-throughput methods. In general, identity should be fixed as early as possible at the upstream stage. Midstream samples require checks from several types of evidence. Downstream products, by contrast, depend more heavily on chemical profiling and task-oriented DNA tools. A stage-specific framework may better match routine quality control and regulatory decisions than a uniform testing model.

Keywords: Chinese Medicinal Plant Materials; Authentication; Supply Chain; Integrative Evidence.

1. Introduction

Chinese medicinal plant materials are widely used in clinical practice and in the manufacture of traditional Chinese medicine products. Their botanical source matters. It affects chemical composition, pharmacological activity, and safety profile[1-3]. Closely related plants, substitutes, or wrongly used medicinal parts may look acceptable at first, yet their chemistry and toxicity can differ. For this reason, authentication is more than a routine quality-control step. It provides the basis for later evaluation[1, 2].

The problem becomes harder once materials enter a modern supply chain. After collection, a material may undergo primary processing, distribution, decoction-piece preparation, and product manufacture. Along this route, macroscopic characters may be cut away, microscopic structures may be damaged, and DNA may be degraded[4-6]. Meanwhile, substitution, admixture, and mislabeling can appear or accumulate during circulation. Market surveys and reviews have reported that such identity problems still occur in commercial samples [4,5].

Many authentication methods are available. However, they do not all examine the same evidence, nor do they answer the same practical question[6-9]. This review therefore discusses authentication together with the changes that occur along the supply chain. It first addresses multiple botanical sources and taxonomic ambiguity. It then reviews the main methods and, finally, proposes a framework in which methods are selected according to the stage and task of the sample.

2. Multiple Botanical Sources and Taxonomic Ambiguity in the Supply Chain

The difficulty of authenticating Chinese medicinal plant materials is not caused only by limitations in analytical

technology. In many cases, the medicinal name itself is already complicated. A single medicinal material name may correspond to more than one accepted botanical source. Local substitutes, regional customary names, inconsistent nomenclature, and older labels retained after processing can further widen the gap between the declared name and the actual source[1, 10]. These problems have long affected the standardization of Chinese medicines and the interpretation of materia medica records[1, 10].

This mismatch is not merely a naming issue. It also influences chemical evaluation, safety assessment, and traceability. Upstream samples usually retain plant morphology, habitat information, collection records, and, when properly handled, voucher specimens. After processing and circulation begin, much of this context can disappear quickly. Later testing may still detect a problem, but it is often difficult to recover the same level of taxonomic certainty[1, 6]. Thus, botanical ambiguity continues to affect later quality judgments rather than ending at the naming stage.

With intact materials, morphological and taxonomic assessment has a clear practical value. It can link the plant individual, the medicinal part, and the source record in a direct way. In midstream and downstream samples, the question changes. Researchers must ask whether the material in hand still agrees with its declared identity, whether substitution or admixture has occurred, and whether any deviation is likely to affect quality. For this reason, authentication should not be reduced to a single method. The evidence needs to be arranged around the sample stage.

3. Major Authentication Methods and Their Scope of Application

3.1. Changes in Available Evidence and Methodological Differences

Once materials move through the supply chain, the

available evidence changes. In relatively intact samples, external morphology and internal structure can still be observed. With deeper processing, those structural clues become weaker. Chemical profiles and molecular traces then carry more weight. The difference among authentication methods is therefore more than a matter of laboratory procedure. Each method reads a different layer of evidence.

3.2. Morphological and Microscopic Methods

Morphological authentication observes external characters of the medicinal material. Microscopic authentication, by contrast, examines tissue- and cell-level traits[6, 7, 11].

Morphology remains useful because it is direct, rapid, and inexpensive. It is especially suitable for whole plants, raw medicinal materials, and lightly processed samples. Microscopy can still help after the external appearance has changed. Vessels, fibers, stone cells, crystals, and starch grains may remain visible and provide diagnostic clues. For that reason, microscopic examination keeps practical value in processed slices and in some powders[7, 11].

Both methods have a similar weak point. They rely on recognizable structural information. When a material is finely powdered, heavily processed, or mixed into a multi-component preparation, that information becomes sparse and interpretation becomes less secure. In practice, morphology and microscopy are most reliable as basic evidence in upstream and midstream work[6, 7].

3.3. Chemical and Spectroscopic Methods

Chemical analysis shifts attention from structure to composition. HPTLC, HPLC, GC-MS, and chromatographic fingerprinting are often used to compare characteristic constituents or whole profiles. These data help judge whether a sample agrees with its declared identity and expected quality status[8].

This kind of evidence is valuable when the sample has lost much of its visible structure. It can be applied to crude drugs, processed slices, and some processed materials. In routine work, chemical methods are particularly useful for batch comparison, quality-consistency assessment, and the screening of unusual samples[8]. Spectroscopic methods, including near-infrared, infrared, and Raman spectroscopy, usually capture overall signals and then use chemometric models for rapid classification[12, 13].

At the same time, chemical evidence has limits. A reasonable compositional profile does not always prove full botanical identity. Closely related species may share many constituents, and processing can change constituent abundance. Spectroscopic results also depend strongly on the training set and model construction. Therefore, chemical and spectroscopic methods are better used as supporting evidence within an integrated assessment[8, 12, 13].

3.4. DNA-Based Methods

DNA-based methods infer biological origin from genetic sequences. DNA barcoding was proposed in 2003, and plant barcoding systems were developed afterward. Among common markers, ITS2 has been widely applied in the identification of medicinal plants[14-18].

These methods are useful because they do not require intact external morphology. They can help with samples that are difficult to distinguish by appearance, with closely related taxa, or with materials from uncertain sources[9, 19]. Conventional DNA barcoding is more suitable for one species

or for a small group of source species. Mini-barcoding improves the amplification of degraded samples by using shorter target fragments. Metabarcoding is better suited to complex mixtures. qPCR and ddPCR are more appropriate when the target is a specific material that needs detection or quantification[9, 20-23]. Shotgun sequencing, target capture, and super-barcoding further expand the options for complex samples and closely related taxa[24-26].

These advantages do not remove the basic constraints. DNA methods still require usable genetic material, and the result depends on database quality, workflow control, and contamination management. In complex products, a DNA trace does not necessarily reflect the true proportion of a component. Results should therefore be interpreted in relation to the analytical task, rather than read as stand-alone proof [9, 19, 20, 27].

3.5. Emerging Technical Approaches

DNA-based authentication has continued to develop in recent years. LAMP, BAR-RPA, Bar-HRM, microfluidic platforms, and digital PCR have mainly been used to make amplification faster, detection more targeted, or workflows simpler [27].

Their value is mainly practical. They can shorten testing time, reduce some operational steps, and, in certain settings, support low-cost screening or on-site testing [27].

However, their application targets and robustness are still uneven. Standardized use also remains limited across different materials and laboratories. At this stage, these techniques are better regarded as supplementary screening tools, rather than replacements for established authentication systems.

4. Stage-Specific Framework for Method Integration

4.1. Basic Approach

No single protocol can fit every stage, sample type, and analytical task. Method selection should begin with the position of the sample in the supply chain. It should also consider the question being asked and the depth of interpretation required. In this sense, a workable framework is not built by simply adding more techniques. It is built by placing each method where its evidence is most useful.

4.2. Upstream Stages

During cultivation, collection, and initial identification, the material is usually still intact. Morphological information, medicinal parts, and source records are also best preserved at this point. The upstream stage is therefore the most suitable time to fix identity, mainly through morphology, taxonomy, and voucher specimens [1, 6]. DNA barcoding can serve as confirmatory evidence, especially for closely related species, incomplete specimens, or high-value materials prone to substitution. It should supplement, rather than replace, the initial taxonomic assessment [9, 19].

4.3. Midstream Stage

Raw medicinal materials, processed slices, and routinely traded samples occupy a more complicated midstream position. Intact plants are no longer available, and batch variation, grade differences, or source admixture may become more visible. This stage calls for cross-checking. Morphology can provide first-line screening. Microscopy can support

structural confirmation. Chemical analysis is better suited to batch variation and quality-status evaluation [7, 8]. Conventional DNA barcoding may be added when morphology and microscopy cannot resolve taxonomic ambiguity [9, 19].

4.4. Downstream Stage

In powders, extracts, and finished multi-component products, structural evidence is greatly reduced. Chemical analysis usually becomes the main approach, because characteristic constituents and overall profiles may still be measured after extensive processing [8, 28]. Still, chemical analysis cannot answer every question. If the task is to identify components in a complex sample, metabarcoding or shotgun sequencing is more suitable [20, 24]. If the aim is to confirm a high-risk species or a known adulterant, qPCR and ddPCR are more targeted [22, 23]. When the question still concerns a single botanical source, mini-barcoding is often more practical [21].

4.5. Identification Principles and Practical Limitations

The method combination should follow the authentication task. Taxonomic confirmation requires resolution and identity verification. Adulteration and substitution testing requires anomaly detection followed by targeted validation. Complex-mixture analysis needs broader compositional coverage. Batch-consistency evaluation and batch release, however, require stable and representative results.

Several practical factors also limit application. These include database quality, taxonomic validation, sample representativeness, contamination control, cost, and throughput [9, 20, 27]. In complex samples, evidence often starts from the individual sample. Regulatory decisions and batch release usually require a conclusion at the batch level. Sampling design and interpretation rules therefore remain essential.

Recent studies support this integrated view. DNA metabarcoding and HPTLC can be used together to provide both compositional information and chemical-marker information. A three-tier strategy also shows that barcoding, metabarcoding, and standardized chromatographic analysis can be arranged step by step. In that way, qualitative and quantitative information accumulates progressively [28, 29].

5. Conclusion

Authentication of Chinese medicinal plant materials should not be treated as one uniform test with a fixed pathway. As materials move from collection to processing, trade, and final products, the basis for identification is weakened and transformed. Early in the chain, the plant body, anatomical features, and source records can still provide relatively direct evidence. Once the material is cut, mixed, powdered, or further manufactured, those cues are no longer enough. Authentication then has to rely on other forms of evidence, often indirect and conditional.

For this reason, the key issue is not to select one best technique for all situations. The more important task is to decide which evidence remains usable at a given stage. Morphological and microscopic examination still play a central role when structural traits are preserved. Chemical and spectroscopic methods become more useful when the task shifts to compositional comparison, anomaly screening, or

consistency assessment. DNA-based approaches add capacity for degraded materials, closely related taxa, and mixed samples. Even then, their conclusions are shaped by sequence quality, reference data, laboratory control, and interpretation strategy.

This review therefore supports a stage-oriented way of thinking. Authentication becomes more reliable when methods are chosen according to sample condition and practical purpose. It is also stronger when different lines of evidence are assembled into a connected judgment instead of being read separately. Under current supply-chain conditions, such an approach is more realistic for traceability, more useful for quality assurance, and more meaningful for the safe use and regulation of Chinese medicinal plant materials.

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