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**Review Article**

# A Comprehensive Review of Pharmacognostic Techniques for the Identification and Standardization of Herbal Drugs

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ABSTRACT

The global resurgence in the use of herbal medicines underscores the need for rigorous scientific validation and standardization of plant-based products. Pharmacognosy, a critical discipline within pharmaceutical sciences, offers a systematic framework for the identification, authentication, and quality assessment of medicinal plants through morphological, anatomical, physicochemical, and phytochemical analyses. This review provides a comprehensive overview of classical and modern pharmacognostic techniques used in the standardization of herbal drugs. Initial macroscopic and organoleptic evaluations, followed by detailed microscopic analysis including powder and tissue microscopy, are foundational in determining authenticity. Physicochemical parameters such as ash values, extractive values, and moisture content play a vital role in detecting adulteration and ensuring quality. Advanced techniques such as chromatography (TLC, HPTLC, HPLC), spectroscopy (UV-Vis, FTIR, NMR), and DNA barcoding have significantly enhanced the precision of plant identification and metabolite profiling. The integration of molecular tools with traditional pharmacognostic practices further strengthens the quality control process, facilitating the development of standardized herbal formulations. Challenges such as regional variability, environmental influences, and lack of uniform global standards persist, yet emerging technologies like artificial intelligence, metabolomics, and portable diagnostic tools offer promising solutions. This review emphasizes the importance of combining multidisciplinary approaches to ensure the safety, efficacy, and reproducibility of herbal medicines, promoting their acceptance in mainstream healthcare systems worldwid

## Introduction

Herbal medicines have been an integral part of human healthcare for centuries, forming the foundation of many traditional systems such as Ayurveda, Traditional Chinese Medicine (TCM), and Unani. In recent decades, there has been a global resurgence of interest in plant-based remedies, not only due to their cultural and historical relevance but also because of the growing demand for alternative and complementary therapies that are perceived as safer and more holistic (Ekor, 2014; Pan et al., 2014). According to the World Health Organization (WHO), approximately 80% of the population in developing countries relies on traditional herbal medicines for primary healthcare needs (WHO, 2013).

Despite their widespread use, the quality, safety, and efficacy of herbal drugs remain a major concern. One of

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**Table 1:** Common Pharmacognostic Parameters Used in Medicinal Plant Identification and Standardization

| *Parameter* | *Type* | *Significance* |
|---|---|---|
| Macroscopic characters | Morphological | Helps in preliminary identification based on color, odor, taste, and texture |
| Microscopic characters | Anatomical | Confirms structural features like stomata, trichomes, xylem, and phloem tissues |
| Ash value (total/sulphated) | Physicochemical | Indicates purity and presence of inorganic matter |
| Extractive values | Physicochemical | Reflects solubility in water/alcohol; used for quality estimation |
| Fluorescence analysis | Diagnostic | Helps detect plant constituents using UV light-based reactions |
| Chromatographic profiling | Phytochemical | Used for identification and fingerprinting of chemical constituents |
| DNA barcoding | Molecular | Confirms genetic identity and detects adulterants or substitutes |
| Spectral techniques (FTIR, NMR) | Analytical | Aids in structural elucidation of phytoconstituents |

the most critical challenges is the accurate identification and standardization of medicinal plants, which is essential to prevent adulteration, substitution, and variability due to geographic and environmental factors (Patwardhan et al., 2005; Heinrich, 2015). Pharmacognosy, the study of medicinal drugs derived from natural sources, plays a central role in this context by providing scientific tools and methodologies for the authentication, evaluation, and quality control of herbal drugs (Mukherjee, 2019).

Pharmacognostic evaluation typically involves a combination of morphological, anatomical, physicochemical, and phytochemical analyses, with recent advancements including molecular markers and spectroscopic profiling. This review aims to provide a comprehensive overview of the classical and modern pharmacognostic techniques employed in the identification and standardization of medicinal plants, highlighting their applications, limitations, and future potential in herbal drug development.

## Historical Evolution of Pharmacognosy

Pharmacognosy, derived from the Greek words *pharmakon* (drug) and *gnosis* (knowledge), has evolved from a traditional art of plant-based healing into a multidisciplinary scientific discipline. Its historical roots can be traced back to ancient civilizations where traditional medical systems like Ayurveda in India, Traditional Chinese Medicine (TCM), and Unani in the Middle East documented the use of plant materials for healing purposes (Kumar et al., 2017; Zhang et al., 2015). These systems emphasized holistic approaches and utilized elaborate materia medica texts detailing hundreds of medicinal plants, their properties, and therapeutic applications.

In ancient India, texts such as the *Charaka Samhita* and *Sushruta Samhita* provided detailed botanical descriptions and formulations (Sharma, 2001). Similarly, the Chinese *Shennong Bencao Jing* and the Islamic *Canon of Medicine* by Avicenna are monumental contributions that laid early pharmacognostic foundations (Liu et al., 2018; Gutas, 2001). These traditional systems employed sensory evaluation—taste, texture, aroma, and appearance—as preliminary diagnostic tools for plant identification and therapeutic categorization.

The scientific transformation of pharmacognosy began during the Renaissance and Enlightenment periods, when naturalists and apothecaries in Europe began to systematically catalog and classify medicinal plants using microscopic features and taxonomic systems (Evans, 2009). The discovery of active constituents such as morphine (1805), quinine (1820), and atropine (1833) marked the shift from crude herbal use to isolation of bioactive compounds, paving the way for modern phytochemistry (Sneader, 2005).

With the advent of analytical chemistry in the 20th century, pharmacognosy integrated physicochemical techniques like chromatography, spectroscopy, and later molecular biology, allowing for precise plant identification, authentication, and quality control (Heinrich et al., 2004). Today, pharmacognosy blends ethnobotanical knowledge with modern scientific techniques, contributing significantly to drug discovery, herbal standardization, and integrative medicine. Table 1 summarizes the common pharmacognostic parameters used in medicinal plant identification and standardization:

## Macroscopic and Organoleptic Evaluation

Macroscopic and organoleptic evaluation represents the initial and most fundamental step in the pharmacognostic analysis of medicinal plants. These methods rely on sensory characteristics such as color, shape, size, odor, taste, and texture, which are crucial for the preliminary identification and quality assessment of crude drugs (Wallis, 2005; Brendler et al., 2022). While simple, these evaluations can be remarkably effective in detecting gross adulteration, substitution, or spoilage, especially in field settings or regions with limited analytical infrastructure. Color and surface characteristics help identify plant parts like roots, leaves, or bark, while odor and taste serve as rapid diagnostic features influenced by the presence of essential oils, alkaloids, or glycosides (Kokate et al., 2019). For example, the pungent odor of *Zingiber officinale* (ginger) or the bitter taste of *Andrographis paniculata* are distinct traits aiding identification. Moreover, texture, such as fibrousness or mucilaginous nature, also contributes to distinguishing between species (Sharma & Sharma, 2020). Organoleptic properties often reflect the phytochemical composition and can suggest therapeutic potential. However, these methods are inherently subjective and may be influenced by the evaluator's experience, environmental conditions, and storage duration (Rafieian-Kopaei et al., 2014). As a result, while macroscopic evaluation is indispensable for initial screening and ethnobotanical fieldwork, it must be complemented by microscopic, physicochemical, or molecular analyses for conclusive authentication and standardization.

Nevertheless, these sensory evaluations remain vital in traditional medicine and are increasingly integrated into modern pharmacognostic protocols as an essential first-line quality control measure (Tosun et al., 2021).

## Microscopic and Histological Techniques

Microscopic and histological evaluations are critical components of pharmacognostic studies, providing anatomical and cellular-level insights that aid in the accurate identification and quality assessment of medicinal plants (Figure 1). These techniques help distinguish plant species and detect adulteration by examining cellular structures such as stomata, trichomes, xylem, phloem, calcium oxalate crystals, and starch grains (Pandey et al., 2018; Pal & Shukla, 2021).

### *Anatomical marker*

such as the type and distribution of stomata (anisocytic, paracytic), the presence of covering or glandular trichomes, and the arrangement of vascular bundles serve as taxonomic features. These are especially useful in differentiating morphologically similar species, as in the case of *Asparagus racemosus* and its substitutes (Ansari & Ali, 2022). Likewise, powder microscopy—a technique involving the examination of powdered crude drugs under light microscopy—reveals diagnostic features like fiber fragments, scalariform vessels, and lignified cells (Bhadane et al., 2017).

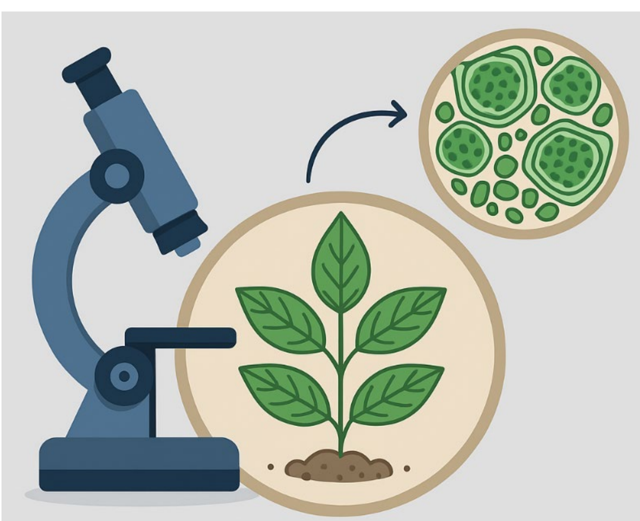

**Figure 1:** Microscopic studies in pharmacognosy

### *Histological staining*

plays a significant role in localizing and identifying bioactive constituents within plant tissues. Specific stains such as phloroglucinol-HCl for lignin, Sudan III for lipids, and iodine for starch granules help verify the phytochemical distribution and confirm the identity of herbal drugs (Singh et al., 2020). These visual tools are especially vital when dealing with processed or dried materials, where macroscopic features are obscured.

The integration of digital microscopy and imaging software has further enhanced the precision of histological observations, allowing for the documentation and comparative study of plant microanatomy (Verma & Singh, 2019). In modern pharmacognosy, microscopic techniques not only support botanical authentication but also contribute to the development of pharmacopoeial standards and regulatory frameworks.

## Physicochemical Analysis

Physicochemical analysis is a vital aspect of pharmacognostic studies, providing quantitative benchmarks for assessing the purity, quality, and identity of herbal raw materials. These parameters are standardized in official pharmacopoeias and are essential in establishing reproducibility, detecting adulterants, and ensuring therapeutic efficacy (Mukherjee, 2019; World Health Organization [WHO], 2022).

### *Ash values*

(total ash, acid-insoluble ash, and water-soluble ash) reflect the total amount of inorganic residues remaining after incineration and help detect impurities such as sand, soil, or metallic salts. A high acid-insoluble ash value, for instance, indicates contamination with siliceous materials, a common sign of adulteration (Khandelwal, 2018).

### *Extractive values*

(water and alcohol soluble) represent the proportion of constituents extractable in specific solvents, suggesting the presence of polar and non-polar phytochemicals. These values are significant for estimating the active content and are used to screen plant materials with therapeutic potential (Patil et al., 2020).

### *Moisture content*

or loss on drying is crucial for determining the shelf life of herbal materials. High moisture levels promote microbial growth and enzymatic degradation, compromising both safety and potency (Bagul et al., 2019).

*pH values*

also serve as critical markers, particularly for formulations where pH-sensitive constituents may degrade or precipitate. Monitoring pH helps in maintaining formulation stability (Bora & Das, 2021).

Regulatory bodies such as the Indian Pharmacopoeia (2022), British Pharmacopoeia (2021), and United States Pharmacopeia (USP, 2023) outline standardized procedures and permissible limits for each parameter. These physicochemical profiles serve as baseline quality control tools and support the standardization of herbal medicines across global markets.

## Phytochemical Screening and Metabolite Profiling

Phytochemical screening and metabolite profiling are integral to pharmacognostic evaluations, as they reveal the presence and relative abundance of bioactive secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and saponins (Harborne, 1998; Tiwari et al., 2011). Preliminary phytochemical screening provides a foundation for identifying therapeutic potentials and guiding advanced analytical investigations.

*Qualitative chemical tests*

employ specific colorimetric or precipitation reactions—such as Dragendorff's for alkaloids, Shinoda for flavonoids, and ferric chloride for phenolics—to detect major classes of phytochemicals (Evans, 2009). These tests, while simple, remain crucial for confirming the chemical nature of crude extracts.

*Chromatographic techniques*

enhance the resolution and specificity of phytochemical detection. Thin-layer chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) are widely used for fingerprinting crude extracts. HPTLC offers reproducibility and densitometric quantification of marker compounds, thereby supporting standardization (Chanda, 2014; Yadav & Agarwala, 2011). High-performance liquid chromatography (HPLC) and gas chromatography (GC) provide detailed separation and quantification, especially useful for phenolics, alkaloids, and essential oils (Pandey et al., 2016; Llorent-Martínez et al., 2020).

*Spectroscopic methods*

such as UV-Vis, FTIR, NMR, and Mass Spectrometry further deepen the chemical understanding of plant constituents. FTIR identifies functional groups, while NMR and MS elucidate molecular structures, enabling dereplication and novel compound discovery (Sharma et al., 2021; Cieśla et al., 2012).

*Metabolomics and chemometric approaches*

including principal component analysis (PCA) and hierarchical clustering, have revolutionized plant analysis by enabling data-driven interpretation of complex phytochemical datasets. These tools facilitate classification of plant species, detection of adulterants, and quality control of herbal drugs (Sasidharan et al., 2011; Rutz et al., 2020).

Together, these techniques enable a comprehensive understanding of plant biochemistry, support authentication, and ensure the reproducibility of herbal formulations.

## DNA Barcoding and Molecular Authentication

DNA barcoding has emerged as a reliable tool for the authentication of medicinal plants, particularly in cases where traditional macroscopic and microscopic methods fall short due to processed or powdered material forms (Chen et al., 2010). It involves the use of short, standardized gene regions to identify plant species based on genetic divergence, offering high precision and reproducibility.

Commonly used genetic markers include the nuclear ribosomal internal transcribed spacer (ITS) region, plastid genes such as matK, rbcL, and trnH-psbA, which show high interspecific variability (Hollingsworth et al., 2011; Fazekas et al., 2012). These regions serve as molecular signatures and can effectively distinguish even closely related species in complex herbal mixtures.

*PCR-based techniques*

such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), and Amplified Fragment Length Polymorphism (AFLP), have also been applied for species differentiation and quality assessment of crude drugs (Jin et al., 2006; Joshi et al., 2004). Among them, ITS sequencing combined with real-time PCR has shown great promise for detecting adulteration in herbal formulations (Zhao et al., 2021).

Compared to conventional pharmacognostic methods, DNA-based techniques are less affected by environmental conditions, plant part, or developmental stage, making them superior for unambiguous identification (Techen et al., 2014). Moreover, DNA barcoding supports regulatory frameworks by facilitating traceability and compliance in global herbal markets (Parveen et al., 2016).

However, challenges remain, such as degraded DNA in processed materials, database limitations, and lack of harmonized international protocols (Sucher & Carles, 2008). Despite these issues, integrating DNA barcoding with chemometric tools and metabolomic profiling can significantly enhance the robustness of plant authentication systems.

## Integration of Pharmacognostic Methods

The effective identification, evaluation, and standardization of medicinal plants necessitate the integration of classical pharmacognostic approaches with modern scientific techniques. Historically, pharmacognosy relied primarily on morphological, microscopic, and organoleptic evaluation for authentication. However, the complexity of global herbal trade and increasing incidences of adulteration now demand more robust, multidisciplinary methodologies (Kumar et al., 2011; Mukherjee, 2019).

Integrating microscopy, phytochemistry, chromatography, and molecular biology enhances the reliability of pharmacognostic studies. For instance, microscopic analysis remains indispensable for identifying unique anatomical features such as stomata, trichomes, and vascular arrangements (Sharma et al., 2020). When combined with HPTLC or LC-MS fingerprinting, these parameters can provide a comprehensive identity and purity profile of plant material (Nikam et al., 2014).

International organizations, particularly the World Health Organization (WHO), have emphasized the need for harmonized monographs and guidelines to support quality control and global trade of herbal medicines. WHO monographs incorporate organoleptic, histological, and phytochemical data, along with purity tests for contaminants such as pesticides and heavy metals (WHO, 2007). Similarly, pharmacopoeias like the European Pharmacopoeia, Indian Pharmacopoeia, and Chinese Pharmacopoeia now mandate integrated methods for plant authentication and standardization (Kunle et al., 2012).

The integration of multiple methods also facilitates the development of monographs, which serve as reference documents for regulatory, clinical, and industrial stakeholders. These protocols help ensure batch-to-batch consistency, efficacy, and safety of plant-derived pharmaceuticals (Patel & Patel, 2021).

Overall, the synergy of traditional knowledge and modern analytical tools forms the foundation of global herbal pharmacovigilance, ensuring both scientific validity and cultural relevance in medicinal plant research.

## Discussion

Despite significant advances in pharmacognostic science, various challenges persist in the accurate identification and standardization of herbal drugs. One major issue is the intentional or unintentional adulteration and substitution of plant materials, which compromises the safety and efficacy of herbal formulations (Zhao et al., 2022). In developing countries, market samples often include morphologically similar but pharmacologically different species, complicating authentication (Pandey et al., 2016).

### Regional and environmental variations

including soil composition, climate, altitude, and harvesting season—significantly affect the morphology and chemical profile of plants. These factors can result in variations in phytoconstituent concentrations, leading to inconsistent therapeutic outcomes (Singh et al., 2015). Moreover, traditional identification methods based on morphology are inadequate when dealing with processed or powdered crude drugs.

Another key challenge is the lack of harmonized protocols across different countries. While some nations follow well-established pharmacopoeial standards, many developing regions lack infrastructure or regulatory oversight for routine quality testing (Upton et al., 2019). Additionally, limitations in access to DNA barcoding infrastructure and reference databases hinder universal implementation of molecular identification tools (de Boer et al., 2017).

The future of pharmacognostic research is being shaped by digital and molecular innovations. Artificial Intelligence (AI) and machine learning algorithms are now being applied to microscopy and image-based plant identification, enhancing accuracy and minimizing human error (Rong et al., 2020). These tools can automate the recognition of microscopic features such as stomatal index or trichome density, offering reproducible results.

Portable spectroscopic devices, such as handheld Raman and NIR spectrometers, are being developed for on-site authentication of herbal raw materials, allowing non-destructive, rapid analysis even in field settings (Wang et al., 2021). This is particularly useful in rural and resource-limited areas.

Furthermore, blockchain technology is gaining attention for its potential in ensuring herbal supply chain transparency. By recording every transaction—from plant collection to final product—in a decentralized ledger, blockchain can prevent adulteration, improve traceability, and ensure regulatory compliance (Tripathi et al., 2023).

Finally, systems biology and omics technologies such as metabolomics, transcriptomics, and proteomics are revolutionizing our understanding of complex plant matrices and their interactions with human biology. These tools can facilitate rational drug discovery from botanicals by identifying active pathways and synergistic compounds (Li et al., 2020).

## Conclusion

Pharmacognostic studies remain the cornerstone for the proper identification, authentication, and standardization of medicinal plants, which are integral to traditional and modern herbal medicine systems. This review has highlighted the significance of integrating classical techniques—such as macroscopic and microscopic analysis—with advanced technologies including chromatography, spectroscopy, molecular markers, and AI-based image recognition. Such multidisciplinary approaches enhance the reliability and reproducibility of herbal drug evaluations, addressing major challenges such as adulteration, substitution, and phytochemical variability due to environmental influences.

Moreover, the standardization of herbal products is vital for ensuring therapeutic consistency, patient safety, and global trade compliance. While regulatory bodies like WHO and national pharmacopoeias have laid important foundations, a globally harmonized framework is still required. The future of pharmacognostic research lies in adopting innovations such as blockchain for supply chain transparency, omics technologies for systems-level understanding, and portable analytical devices for field-level authentication.

In conclusion, advancing pharmacognostic methods through scientific innovation and policy harmonization will not only strengthen the credibility of herbal medicine but also bridge the gap between traditional knowledge and evidence-based healthcare. The evolution of this field is essential to meet the growing demand for safe, effective, and standardized herbal therapeutics in global healthcare systems.

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