

## FINGERPRINTING PROFILES (HPTLC/HPLC): MODERN TECHNIQUES USED TO ESTABLISH IDENTIFY AND CONSISTENCY OF HERBAL DRUGS

AVULA JYOSTHNA\*, P.MOHAN VIKAS, CHANDU BABU RAO

Priyadarshini Institute of Pharmaceutical Education and Research, 5TH Mile, Pulladigunta, Guntur-522017, Andhra Pradesh, India.

**Article History:** Received: 24 Feb 2026, Revised: 09 Mar 2026, Accepted: 19 Apr 2026

### \*Corresponding Author

Avula Jyosthna

#### Abstract

Phytomedicine, commonly referred to as herbal medicine, constitutes a significant component of traditional and modern healthcare systems worldwide. Nearly 80% of the global population relies on herbal medicines for primary health care needs. Despite their widespread usage and therapeutic importance, concerns regarding quality, safety, efficacy, and reproducibility have necessitated stringent standardization practices. Standardization ensures the authenticity, purity, consistency, and therapeutic effectiveness of herbal formulations. This review comprehensively discusses the various approaches to phytomedicine standardization, including pharmacognostical evaluation, physicochemical analysis, biological evaluation, chemical profiling, chromatographic fingerprinting, spectroscopic techniques, DNA barcoding, and chemometric analysis. Advanced analytical methods such as HPLC, HPTLC, GC-MS, LC-MS, FTIR, NMR, and capillary electrophoresis are critically examined. Additionally, regulatory frameworks, quality control challenges, safety concerns, and stability evaluation methods are addressed. The review highlights the limitations of single-marker standardization and emphasizes the importance of fingerprint profiling combined with chemometrics for holistic quality assessment. The integration of advanced analytical technologies and regulatory compliance is essential to ensure global acceptance and therapeutic reliability of herbal medicines. **Keywords:** Phytomedicine, Standardization, Herbal drugs, Chromatographic fingerprinting, Quality control, Chemometrics, WHO guidelines.

**Keywords:** Lemon, Metabolism, Extraction, Green Chemistry.

This article is licensed under a Creative Commons Attribution-Non-commercial 4.0 International License. Copyright © 2026 Author(s) retains the copyright of this article.



#### INTRODUCTION

Herbal medicine has been used for thousands of years across various civilizations including Ayurveda, Traditional Chinese Medicine, and Unani systems. Medicinal plants serve as a primary source of therapeutic agents, and many modern drugs originate from plant-derived compounds.

Phytomedicine refers to medicinal preparations derived from plants through processes such as harvesting, drying, extraction, purification, and formulation. Herbal materials include leaves, roots, bark, flowers, seeds, rhizomes, resins, oils, gums, and extracts [1].

However, unlike synthetic drugs, herbal medicines contain complex mixtures of bioactive compounds. Variations in geographical location, harvesting time, climate, soil conditions, and processing methods significantly influence chemical composition. Therefore, standardization is essential to ensure consistent therapeutic outcomes.

Standardization may be defined as the process of ensuring batch-to-batch consistency in quality, purity, safety, and efficacy of herbal medicines [2].

#### 2. IMPORTANCE OF STANDARDIZATION

Standardization is necessary to:

Prevent adulteration and substitution, Ensure reproducibility of therapeutic effects, Maintain quality consistency, Reduce toxicity and contamination risks, Facilitate regulatory approval, Enhance global market acceptance

Without proper standardization, herbal products may vary significantly in chemical composition, leading to reduced efficacy or adverse effects.

#### 3.WHO GUIDELINES FOR STANDARDIZATION [3]

The World Health Organization (WHO) has established guidelines for quality control of herbal materials. These include: Botanical authentication, Macroscopic and microscopic evaluation,

Physicochemical analysis, Chromatographic profiling, Safety assessment, Toxicological studies, Stability testing. WHO emphasizes Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP) to ensure quality from cultivation to finished product.

#### 4. METHODS OF HERBAL DRUG EVALUATION

##### 4.1 Organoleptic Evaluation

Drugs can be evaluated organoleptically using the sense organs (skin, eye, tongue, nose, ear, etc.) or microscopically, which includes evaluating the drug based on color, taste, odor, size, shape, and unique characteristics like touch, texture, etc. For instance, the fragrant scent of umbelliferous fruit, the pleasant scent of liquorice, Cinchona and cascara bark have a fractured surface.

Organoleptic analysis helps in primary identification and detection of adulteration

##### 4.2 Microscopic Evaluation [4]

It entails a thorough analysis of medications and can be used to recognize organized pharmaceuticals based on their recognized histological characteristics. Using microscopic analysis, it is primarily utilized for qualitative assessment of crude pharmaceuticals arranged in absolute and potency forms.

Microscopy is used to examine:

Cellular structure, Trichomes, Stomata, Vascular bundles, Starch grains. Important quantitative microscopy parameters include:

Palisade ratio, Stomatal number, Stomatal index, Vein-islet number, Vein termination number, These parameters help differentiate closely related plant species

##### 4.3 Physical Evaluation

Physical Evaluation: When assessing a certain medication, physical constants are occasionally taken into account. These consist of solubility in different solvents, water content, specific gravity, optical rotation, refractive power, melting point, and viscosity. Moreover, the following are included: moisture content, viscosity, pH, disintegration time, friability, hardness, flowability, flocculation, sedimentation, total ash, acid-insoluble ash, water-soluble ash, swelling index, foam index, and sequential extraction value. and the amount of alcohol. These physical characteristics are all helpful in recognizing and detecting plant ingredients

Physical parameters include:

- Ash value (total, acid-insoluble, water-soluble)
- Extractive value (alcohol-soluble, water-soluble)
- Moisture content
- Refractive index
- Specific optical rotation
- Melting point
- Viscosity
- pH

These values indicate purity, identity, and quality of herbal materials.

#### 5. BIOLOGICAL EVALUATION

Biological assays evaluate pharmacological activity.

##### 5.1 Bitterness Value

Used for appetite-stimulating herbs.

##### 5.2 Swelling Index

Measures mucilage content.

##### 5.3 Foaming Index

Indicates saponin presence.

##### 5.4 Pesticide Residue Analysis

Detected using GC-MS and LC-MS techniques.

Bioassays ensure therapeutic activity and safety.

#### 6. CHEMICAL EVALUATION

The pharmacological and biological effects of the majority of medications rely on particular chemical components. a qualitative chemical test to assess the purity and quality of a medication.

Chemical tests identify phytoconstituent, Alkaloids (Dragendorff's, Wagner's tests), Glycosides (Borntrager's test), Carbohydrates (Molisch test), Flavonoids, Saponins, Preliminary phytochemical screening supports quality assessment.

#### 7. ANALYTICAL EVALUATION

There are numerous pharmacopeial monographs available, which are the most practical method for ensuring the quality of herbal medicines. The producer should develop and validate analytical methods in the absence of pharmacopoeial monographs. The best course of action is to adhere closely to the assays or pharmacopoeial definitions of identification, purity, and potency. The use of suitable analytical techniques to ascertain identity, purity, and relative potency is essential to fulfilling all monograph requirements. It offers a multitude of analytical techniques.

##### 7.1 Chromatographic Techniques [5]

HPTLC, HPLC, GC, GC-MS, LC, LC-MS, CE.

These techniques provide qualitative and quantitative profiling.

##### 7.2 HPTLC

Advantages of HPTLC:

- The capacity to examine crude samples with several components.
- Separated spots can be found using sensitive and specific color reagents (Dandruff reagent/Kidde reagent)
- Multiple sample analysis, Visual fingerprinting, Cost-effective
- Suitable for crude extracts
- HPTLC is widely accepted in pharmacopoeias for herbal identification.

##### 7.3 Liquid Chromatography- Mass Spectroscopy (LC-MS):

LC-MS provides

high sensitivity, Molecular weight determination, Isotope pattern recognition, Structural elucidation It is

extensively used in drug development and metabolite profiling.

## 8. MARKER COMPOUNDS [6]

Markers are chemically defined constituents used for quality control.

They may be:

Active markers

Analytical markers

Limitations:

Single-marker approach cannot represent full herbal complexity.

### 8.1. STANDARDIZATION OF HERBAL DRUGS: CURRENT CRITERIA, LIMITATIONS, AND CHALLENGES

Macroscopic and microscopic characterizations, chemical tests, chromatographic fingerprinting, DNA profiling, quantitative determination of specific compounds or markers or a group of compounds, testing for chemical contaminants or microorganisms, and physicochemical tests are the common standardization methods specified by those official guidelines [7].

### 8.2. FINGERPRINT DATA ANALYSIS USING CHEMOMETRIC APPROACH

If the quality markers are not yet determined, the preferred method for assessing the quality of HMs is a combination of fingerprinting and chemometrics. HMs' chemical fingerprints and profiles, as determined by experimental techniques like LC-MS/MS and <sup>1</sup>H-NMR spectroscopy, exhibit both similarities and differences. Samples can be categorized into distinct groups, such as real and contaminated HMs, using these sets of similarities and differences. Nevertheless, a lot of chemical reactions from unidentified components occur in all HMs, which makes data management difficult.

Chemical fingerprinting analyzes complete chemical profiles instead of single markers [8].

Methods include:

HPLC fingerprinting, HPTLC fingerprinting, GC fingerprinting, LC-MS profiling, NMR fingerprinting CE

These techniques provide qualitative and quantitative profiling.

### 8.3 HPTLC

Advantages:

Multiple sample analysis

Visual fingerprinting

Cost-effective

Suitable for crude extracts

HPTLC is widely accepted in pharmacopeias for herbal identification.

### 8.4 LC-MS

LC-MS provides:

High sensitivity, Molecular weight determination, Isotope pattern recognition, Structural elucidation

It is extensively used in drug development and metabolite profiling [9].

## 9. MARKER COMPOUNDS

Markers are chemically defined constituents used for quality control.

They may be:

Active markers

Analytical markers

Limitations:

Single-marker approach cannot represent full herbal complexity [10].

## 10. CHEMICAL FINGERPRINTING

Chemical fingerprinting analyzes complete chemical profiles instead of single markers.

Methods include:

HPLC fingerprinting, HPTLC fingerprinting, GC fingerprinting, LC-MS profiling, NMR fingerprinting  
Fingerprinting ensures comprehensive quality assessment.

Analytes in herbal samples are ionized or desorbed in DESI by applying charged solvent droplets to the sample surface. Samples can be analyzed using this method without any prior preparation. The geographical distribution of secondary metabolites within the sample specimen can be mapped using surface imaging made possible by DESI [10, 11]. (Freitas et al., 2019). In addition to dosage forms like tablets and capsules, DESI has been used on raw herbal materials such leaves, stems, roots, and blossoms (Yang and Deng, 2016). Analyte desorption and/or ion transfer to the mass may be hampered by powdered samples' potential to splatter after being struck by solvent droplets, making DESI difficult. Powdered samples can be soaked in a volatile solvent and placed as a thin layer over a solid surface before MS analysis, or they can be compressed into thin tablets to get around this issue [12].

### 10.1. CHEMOMETRICS IN HERBAL STANDARDIZATION

Chemometrics applies statistical tools to analyze complex chemical data.

Common techniques:

PCA (Principal Component Analysis), PLS-DA

Statistical parameters:

R<sup>2</sup> (Coefficient of determination), RMSE (Root Mean Square Error), Q<sup>2</sup> (Predictive ability)

Chemometrics enhances classification and authentication accuracy [13].

## 11. DNA FINGERPRINTING AND BARCODING

In order to provide a distinct pattern for identification, DNA fingerprinting is a technique that concurrently detects mini- or microsatellites, which are little stretches of repeated DNA that exhibit larger variances among individuals. Particularly helpful for plant genotyping and managing the quality of medicinal crops are DNA fingerprinting techniques (Zhokhova et al., 2019). Finding plant varieties with desirable qualities could help prepare more uniform plant materials with similar characteristics since differences in the genetic

materials of plants may affect the phenotypes, including the production of secondary metabolites.

DNA-based techniques ensure botanical authentication.

Methods:

RAPD, AFLP, ISSR, SNP, STR, VNTR

Advantages:

- Detects adulteration
- Independent of environmental factors
- Useful for processed materials
- Limitations include cost and technical expertise requirements.

## 12. CHALLENGES ASSOCIATED WITH QUALITY CONTROL OF HERBAL FORMULATIONS AND MATERIALS PRODUCT

The majority of herbal product labels frequently don't tell you everything that's in the bottle. According to studies, there are notable discrepancies between what is indicated on the label and what is in the bottled items, and consumers have less than a 50% probability of actually receiving what is listed on the label. Due to the lack of a legal meaning, the term "standardized" on a product label does not necessarily indicate that the product is of superior quality. Customers are frequently burdened with determining what is safe and beneficial for them, and their irritation is further increased by the inconsistent labeling of herbal goods.

### Challenges include

Adulteration, Microbial contamination, Batch variability, Inconsistent labeling, Strict regulatory compliance and GMP implementation

## 13. SAFETY EVALUATION

- Herbal medicines may cause:
- Hepatotoxicity
- Nephrotoxicity
- Allergic reactions
- Drug interactions
- Safety evaluation includes:
- Toxicological studies
- Pharmacovigilance
- Heavy metal testing
- Microbial testing
- Robust pharmacovigilance systems are essential

## 14. FUTURE PROSPECTS [14]

Future trends include: Integration of AI in chemometric analysis, Advanced hyphenated techniques, Metabolomics-based standardization, Global harmonization of herbal regulations, Development of universal quality markers, Standardization must move from single-marker evaluation to multi-component fingerprint analysis.

## 15. CONCLUSION

The standardization of phytomedicine is essential to ensure quality, safety, and efficacy. Conventional

pharmacognostical methods combined with advanced analytical techniques such as HPTLC, LC-MS, NMR, and DNA barcoding provide comprehensive evaluation strategies. Chemometric analysis strengthens fingerprint interpretation and classification accuracy.

Although significant progress has been made, challenges remain in marker selection, regulatory harmonization, and global standardization practices. Future research should focus on multi-marker fingerprint approaches and integration of advanced technologies to achieve reliable and reproducible herbal therapeutic

## 16. AUTHOR CONTRIBUTIONS

All authors are contributed equally.

## 17. FINANCIAL SUPPORT

None

## 18. DECLARATION COMPETING INTEREST

The authors have no conflicts of interest to declare.

## 19. ACKNOWLEDGEMENTS

None

## 20. REFERENCES

1. Liang YZ, Xie P, Chan K. Quality control of herbal medicines. *Journal of Chromatography B*. 2004;812(1-2):53-70. doi:10.1016/j.jchromb.2004.08.041.
2. Chopra P, Narish, Sindhu RK. Standardization and evaluation of herbal drugs is need of the hour in present era. doi:10.64105/mttxha32.
3. Shulammithi R, Sharanya M, Tejaswini R, Kiranmai M. Standardization and quality evaluation of herbal drugs. *IOSR Journal of Pharmacy and Biological Sciences*. 2016;11(5-1):89-100. doi:10.9790/3008-11050189100.
4. Hosbas Coskun S, Wise SA, Kuszak AJ. The importance of reference materials and method validation for advancing research on the health effects of dietary supplements and other natural products. *Frontiers in Nutrition*. 2021;8:786261. doi:10.3389/fnut.2021.786261.
5. Muyumba NW, Mutombo SC, Sheridan H, Nachtergaeel A, Duez P. Quality control of herbal drugs and preparations: The methods of analysis, their relevance and applications. *Talanta Open*. 2021;4:100070. doi:10.1016/j.talo.2021.100070.
6. Azlah MA, Chua LS, Abdullah FI, Yam MF. A fast and reliable 2D-IR spectroscopic technique for herbal leaves classification. *Vibrational Spectroscopy*. 2020;106:103014. doi:10.1016/j.vibspec.2019.103014.
7. Crawford EA, Gerbig S, Spengler B, Volmer DA. Rapid fingerprinting of lignin by ambient ionization high resolution mass spectrometry and simplified data mining. *Analytica Chimica Acta*. 2017;994:38-48. doi:10.1016/j.aca.2017.09.012.
8. Hwisa NT, Gindi S, Rao CB, Katakam P, Rao Chandu B. Evaluation of antiulcer activity of

- Picrasma quassioides Bennett aqueous extract in rodents. Vedic Research International Phytomedicine. 2013;1:27.
9. Sumalatha Gindi, Tanuja Methra, Rao Chandu, et al. Antiurolithiatic and in vitro anti-oxidant activity of leaves of Ageratum conyzoides in rat. World Journal of Pharmacy and Pharmaceutical Sciences. 2013;2(2):636-649.
  10. Sreekanth Nama, Chandu BR, et al. Development and validation of a new RP-HPLC method for the determination of aprepitant in solid dosage forms. Tropical Journal of Pharmaceutical Research. 2011;10(4).
  11. Rao AA, Rao CH Babu, Devanna N. Design and evaluation of mucoadhesive buccal bilayered tablets of metoprolol succinate. World Journal of Pharmaceutical Research. 2017;7(3):172-178.
  12. Rani CH Usha, Sumalatha G, Rao CH Babu, Varalakshmi TN. Alzheimer's disease-pharmacotherapeutic interventions. International Journal of Pharmaceutical and Chemical Sciences. 2013;2(2).
  13. Prasanthi G, Chandu Baburao, Pradeep Kumar Y, Swarnalatha D, Gopinath. Chemical pharmacology of khat leaves. Journal of Global Trends in Pharmaceutical Sciences. 2014;5(4):2024-2029.
  14. Hwisa NT, Chandu Babu Rao, Katakam P, Dasari V. Influence of sodium CMC and HPMC on the physical characteristics of ofloxacin floating matrix tablets. British Journal of Pharmaceutical Research. 2013;3(3):508-522.