Journal of Mass Spectrometry

WILEY

Journal of MASS SPECTROMETRY

RESEARCH ARTICLE

Identification of Botanicals Based on Their Mass Spectrum Fingerprints Using Ultra-Performance Liquid Chromatography-Mass Spectrometry

Akhilesh Kumar[1,2] | Dipak Kumar Mishra[1,2] | Sanjeev Kanojiya[1,2]

[1]Sophisticated Analytical Instrument Facility & Research, CSIR-Central Drug Research Institute, Lucknow, Uttar Pradesh, India | [2]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India

Correspondence: Sanjeev Kanojiya (s_kanojiya@rediffmail.com; sanjeev_kanojiya@cdri.res.in)

Received: 6 February 2025 | **Revised:** 17 March 2025 | **Accepted:** 20 March 2025

Funding: This work was supported by the Science & Engineering Research Board (SERB) DST Government of India (YSS/2015/001048).

Keywords: botanicals | chemical barcode | chemical fingerprinting | digital library | mass spectrum fingerprint

ABSTRACT

In the current scenario, herbal raw materials are identified via morphotaxonomy, microscopic pharmacognosy, or DNA barcoding. However, these methods do not reveal their chemical integrity, while plant raw materials play a crucial role in the quality of plant-based medicine. To overcome this limitation, we used a mass spectrometry-based method to identify 30 botanicals. This assay followed a standard operating procedure (SOP) from sample preparation to the reference library's mass spectrum fingerprint (MSFP) search. The MS1 score showed a similarity index between the input data and the reference mass spectrum. A more than 50% MS1 score was the critical threshold for accurately identifying botanicals based on their chemical integrity. Interestingly, the analysis of 30 different plant species yielded no false results. The results were 100% accurate and selective for tested botanical samples. However, we found that the standard deviation of analytical assays and biological replicates was ± 3.5 and ± 6.3 (MS1 score) for all analyzed samples, respectively. Intraspecies variability showed MS1 scores > 50% ± 10, whereas interspecies variability was observed with MS1 scores < 50% ± 10. The MS1 score was observed, dependent on the plant species, ranging from 53.00% (± 2.65) to 89.76% (± 4.08). In addition, the method was tested to see how seasonal and geographical changes affected search results. The MS1 score changed by less than 15%. We simultaneously created a chemical barcode (unique molecular weight sequence) for each plant species to validate search results and ensure the reliable identification of botanicals.

1 | Introduction

Modern analytical techniques like ultra-high-performance liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), and various spectroscopic methods are fundamental tools to identify individual components within complex mixtures [1]. These advancements have significantly enhanced the accuracy and reliability of chemical characterization, enabling comprehensive chemical profiling of diverse substances. Among these methodologies, chemical fingerprinting has emerged as a robust analytical strategy for the discrimination and authentication of phytopharmaceuticals, polyherbal formulations, and herbal raw materials [2]. This method is based on chemical markers, constituents, or compounds present in the substance, resulting in a distinct chemical "fingerprint" or profile that distinguishes it from others. Its far-reaching applications extend to quality control, authentication, and purity verification across multiple industries, including pharmaceuticals, herbal medicine, and food production [3]. By comparing the chemical fingerprint of a sample against established references or standards, it becomes possible to ascertain the authenticity of

the sample or detect potential adulteration. Additionally, chemical fingerprinting finds its footing in environmental monitoring and forensics [4], facilitating the identification and tracing of the origins of samples from various sources. The historical background of plant identification techniques follows a multifaceted trajectory, with ancient civilizations establishing the roots of morphotaxonomy and botanical knowledge [5]. Carl Linnaeus's systematic binomial nomenclature system in the 18th century provided a standardized approach that is still in use today [6], further enhancing plant classification. Before the arrival of photography [7], botanical illustrations emerged as vital tools for plant identification, followed by the development of herbaria, collections of dried plant specimens that enabled meticulous comparative studies [8]. The 19th and 20th centuries witnessed the production of user-friendly field guides for nonspecialists, while microscopic techniques and chemical analysis improved accuracy. In recent decades, molecular techniques, computer-based identification systems, and inhabitant science have revolutionized plant identification [9], allowing for more precise and efficient methods. Furthermore, global collaboration through organizations and databases like The Plant List and GBIF has fostered international cooperation and the standardization of plant nomenclature and identification techniques [10], reflecting the diverse and ever-evolving strategies employed to understand and classify the plant kingdom. Nowadays, herbal medicines (botanicals) are globally important for their health benefits and economic considerations [11, 12]. The safety and efficacy of herbal medicine depend on the chemical integrity of raw materials and the process of manufacturing [13]. However, no standard practices allow us to identify herbal raw materials based on their chemical fingerprints (chemical integrity) with the least human interference [14, 15]. Raw materials used in the herbal products industry suffer from fraud and unethical practices [16]. Therefore, authenticating raw materials and their chemical integrity validation is the first foundation for creating a quality product [17]. Before the invention of fashionable molecular tools (DNA barcoding) [18–23], the only primary methods of herbal raw materials identification were morphological (phenotype) and anatomical descriptions [24, 25], which typically entailed the description of variation for morphological traits by an expert taxonomist and trained technicians through experience. However, this method does not tell us about their chemical integrity. This common practice of raw materials authentication is in decline due to several constraints [26], including misdiagnosis brought on by a lack of high-level expertise, overlooking taxa with cryptic morphology, and inefficiency brought on by incomplete morphological keys for specific life stages. Although genome-based (genotypic) approaches, including DNA barcoding, have advanced plant identification methodologies [18–23], no single, universally applicable technique offers a comprehensive solution for verifying the chemical integrity of herbal raw materials. To address this gap, we introduce a mass spectrum fingerprint (MSFP)-based approach for the identification and authentication of economically important medicinal plant species [27–29]. This modern, rapid, and reliable analytical technique is based on the chemical integrity of plant materials. A web-based digital library of Indian medicinal plants and their metabolites (a bioinformatics tool) was utilized as a reference library of MSFPs. Currently, this MSFP repository includes over 300 medicinal plant species from 104 plant families, comprising more than 1550 ESI (±) MSFPs and about 4800 ESI-MS/MS spectra of plant metabolites. This work is an advancement in the botanicals authentication and quality assurance of medicinal plant raw materials, offering a valuable resource for researchers, regulatory agencies, and the herbal industry.

2 | Experimental

2.1 | Chemicals and Materials

High-purity solvents (LC–MS grade), acetonitrile (Merck Life Science), and methanol (Molychem) were used throughout the study. Finar, Ahmedabad, Gujarat (India), provided analytical grade ammonium acetate, while ultra-pure water was produced by the Milli-Q system (Merk Millipore, Bangalore, India).

2.2 | Selection of Raw Materials

High-value plant materials with therapeutic qualities that are sold locally were selected for analysis. These plants have a high market value in India and are important to the healthcare and herbal industries. The raw materials of those plants often serve as valuable resources for traditional medicine, pharmaceuticals, and the herbal products industry, and they were selected for the study.

2.3 | Sample Collection and Preparation

Plant raw materials were sourced from the Lucknow region (26.8467°N 80.9462°E) from upper Gangetic plains and also purchased from the local market. *Terminalia chebula* and *Bergera koenigii* samples were provided by the botany unit from different locations to find out their geographical variation on the MS1 score. The plant materials were authenticated by an angiosperm taxonomist (DKM) before being used for analysis. A detailed description of plant materials and their commercial importance is described in Table S1. The obtained plant raw material was dried in a hot air oven at 37 °C and ground into fine powder with a blender. The extraction of raw material (0.5 g of fine powder) was done with 5 mL of methanol at room temperature for 48 h, with 5 min of sonication after 8 h. Subsequently, the methanolic extract was filtered using a 0.22-μm syringe filter and transferred into a sample vial for LC–MS analysis.

2.4 | Data Acquisition

Mass spectrometry (MS) analysis was performed on a hyphenated UHPLC–ESI-MS/MS triple quadrupole (Waters Co., Milford, MA, USA) system. The mobile phase consisted of CH_3CN (A) and 5 mM $NH_4CH_3CO_2$ buffer (B) passed through a Waters BEH C-18 (150 × 2.1 mm, 1.7 μm) column with an applied constant flow rate of 0.250 mL/min using a linear gradient (Table S2). The column and autosampler temperatures were set at 35 ± 2 and 25 ± 5 °C, respectively. The UHPLC eluent was subjected to the mass spectrometer using an electrospray ionization (ESI) source operated in the ES ± ion mode. High-purity N_2 was used as a nebulizing and drying gas at 50 and 650 L/h flow rates, respectively. The ESI source parameters were capillary (3.5 kV) and cone (30 V). The source and desolvation

temperatures were set at 120°C and 350°C, respectively. The raw data were acquired in centroid mode with a scan range of *m/z* 150–1500. Argon was used as a CID gas, and MassLynx (vs. 4.1) software was used for data acquisition and processing.

2.5 | Data Processing and Transformation

To analyze the raw LC–MS data, the file was opened in MassLynx (v4.1), and the Base Peak Ion (BPI) chromatogram was located in either electrospray negative (ES-) or positive (ES+) mode. The "spectrum combine" function was used to select a range by dragging from the last to the first eluted peak on the BPI chromatogram, excluding any background before and after the selected range. This process generated a complete average mass spectrum, termed the MSFP. The resulting peak list was copied and pasted into a Notepad file (a scripted text file) and saved. The saved file was then transformed into TMSD format, which served as input data for the database search, as illustrated in Figures S2-S3.

2.6 | Database Search

Access the https://plantmetabolome.cdri.res.in website and undergo the registration process. After completing the necessary verification via email, use the provided login credentials (login ID and password) to access the mass spectrometry-based search (Figure S4). "Advanced Search Option" was selected, and the notepad (TMSD format) file was browsed, keeping the top three results with a 5% tolerance of relative intensity. After completion of the search process, a window displays the search results (Figure S4O).

3 | Results and Discussion

3.1 | MSFP

The term "fingerprint" in mass spectrometry describes the unique pattern of peaks in a mass spectrum associated with a particular compound or set of compounds. This pattern can serve as a distinctive identifier like a fingerprint, allowing for identifying specific substances. Moreover, the MSFP also represents the chemical characteristics of the analyzed botanical extract or samples. Thus, a "chemical barcode" was planned to be used to identify botanicals. Out of 30 analyzed plant species, six representative MSFPs are shown in Figure 1. It showed that the fingerprints of *T. chebula* and *Terminalia bellirica* were entirely different, while they are taxonomically and chemically closely related species. In the same way, the dominance of chebulic acid (*m/z* 357) can be seen in *T. chebula* as compared to *T. bellirica*. Apart from this, Figure 1a,b shows the characteristic signals or patterns of unidentified peaks at *m/z* 315, 440, 498, 584, 701, and 331, indicating a unique pattern specific to *Andrographis paniculata*, as well as the presence of *m/z* 342 (magnoflorine), 357 (merisperine), 277 (alphalinoleic acid), and 341 (disaccharide sugar) in Tinospora cordifolia. *Bergera koenigii* indicated the presence of carbazole alkaloid peaks at *m/z* 324 (koenimbidine), 348 (mahanine), and 330 (mahanimbine). Beside this, represented (ES+) *m/z* 274, 340, 453, 625, 679, and (ES-) *m/z* 227, 361, 471, 623, 723 characteristic metabolites signal of *Cinnamomum tamala*. Moreover, all the analyzed plant species were shown to have distinctive chemical fingerprints (MSFP) from each other (Figures S5-S8). Accordingly, by capturing the unique pattern of mass-to-charge ratios in a mass spectrum associated with a particular metabolite, MSFP enables the identification of substances or particular plant products. On the other hand, due to its unbiasedness, MSFP has the potential to become a standard methodology in modern analytical chemistry.

3.2 | Identification of Botanicals

Traditionally, identification of herbal raw materials with their chemical integrity is almost difficult for the buyers and sellers. Even botanists use different plant morphological characteristics as identification keys, which are examined sequentially and adaptively to identify plant species. However, it does not tell about their chemical integrity, even by DNA barcoding. Besides this, chemical fingerprinting not only identifies the plant species but also allows confirmation of the chemical integrity of herbal raw material. Therefore, we validated the analytical method for rapid identification of economically important botanicals using MSFP. The SOP was followed, as shown in Figure 2, and validation results are shown in Table 1 with respect to accuracy, reproducibility, MS1 score, and hit (matched *m/z* values). Remarkably, the results were exceptionally accurate across the top three results for each tested plant sample. It is also observed that each plant species test result provided 100% selectivity in both ES (±) MSFP. A comprehensive data set of search results is shown in Table S4, and the results displayed in Figure S4O describe information about plant name, family, metabolites, data coverage, MS1 score, and hits. The data coverage described the extent to which the *m/z* value aligned with the database's top 75 most intense peaks and provided a comparative overview (Figure S4P) of the search results. MS1 score and hit were calculated using the following formula.

$$^{*}\text{MS1 Score}\,(\%) = \{\text{Hit (matched } m/z)\} / \{\text{Total } m/z\,(75)\} \times 100$$

The top three MSFP matches resulted in the average ESI ± MS1 score. Further, it was also found that the *m/z* 200 to 750 was more selective in terms of unique fingerprints or especially important in finding distinct metabolite signals. Additionally, authentication of botanicals can be done by chromatographic fingerprints at distinct wavelengths (220, 240, 280, 350, and 410 nm) and BPI chromatograms as shown in Figure 3. It further enhanced the reliability and accuracy of the plant species identification.

3.3 | Effect of Common Ion Interference

Primary metabolites are universal and common across plants as compared to secondary metabolites unique to particular plant species. The presence of primary metabolites, such as carbohydrates, small organic acids, fatty acids, and chlorophyll, poses a challenge in achieving selective identification of botanicals based on their chemical fingerprints. Therefore, to enhance the selectivity, it was essential to eliminate these common ions. The removal of these interfering ions resulted in a decrease in the MS1 score but enhanced the selectivity due to the characteristic signals of secondary metabolites of a particular plant species. The observed

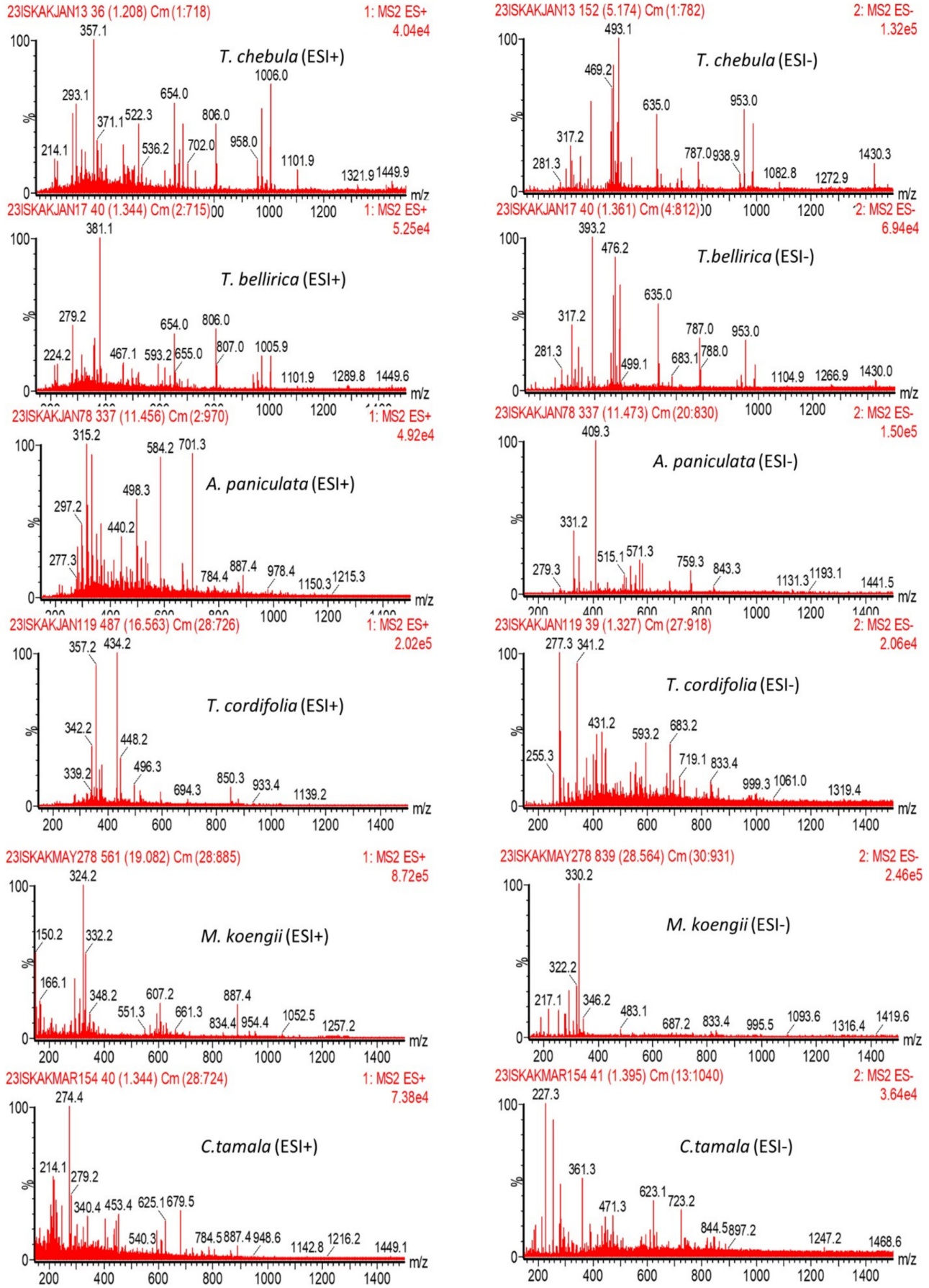

FIGURE 1 | Representative mass spectrum fingerprints of *Terminalia chebula*, *Terminalia bellirica*, *Andrographis paniculata*, *Tinospora cordifolia*, *Bergera koenigii*, and *Cinnamomum tamala* *a = positive mode, b = negative mode.

common ions were detected as (ES+) *m/z* 381, 151, 214, 177, 195, 179, 205, 888, 887, and (ES-) *m/z* 341, 175, 179, 255, 279, 281, 283, 277. The effect of common ion interference on search results was studied in the case of *Mangifera indica*, *Cassia fistula*, and *Azadirachta indica*, and their results are shown in Tables S5–S7.

3.4 | Method Validation

The analytical method and SOP were validated through multiple studies, including analytical and biological reproducibility and the effect of seasonal and geographical variations on search results. The multilayered validation approach enhances the reliability of analytical results with respect to accuracy and reproducibility.

3.5 | Effect of Sample Processing

For the extraction of secondary metabolites from the plant materials, multiple solvents were used according to their polarity in three plant matrices (*A. indica*, *C. fistula*, and *A. marmelos)*. The investigations revealed that the methanolic extract consistently yielded superior results (Tables S8–S10) compared to those obtained from another solvent (water, acetonitrile, ethanol, chloroform, butanol, dichloromethane, ethyl acetate, and hexane) extract even after multiple repetitions.

3.6 | Analytical Reproducibility

Triplicate injections (Figure S9) of each plant sample were injected to assess instrumental reproducibility (Table S11). The standard deviation in the MS1 score was observed as SD ± 3.46 ($n = 3$), while ± 0.50 in the case of data processing (Table S12). Apart from this, the effect of C-18 column length (150, 100, and 50 mm) was also tested for chromatographic and MS1 score reproducibility (Table S13). Across the analytical reproducibility test, the observed standard deviation was less than 3.5 consistently.

3.7 | Biological Reproducibility

It was evaluated by six biological replicates ($n = 6$) of plant samples (Figure S10) collected from different locations in Lucknow, India. The result was 100% accurate with an MS1 score SD ± 6.09 across all samples (Table S14).

3.8 | Inter and Intra Species Variability

Intraspecies maximum variability was observed in the case of *A. paniculata*, which showed MS1 scores > 50% ± 10 across all tested samples. It was also dependent on particular plant species (Table 1), while interspecies variability (Table S15) was lower than intraspecies variability with MS1 scores < 50% ± 10.

3.9 | Effect of Seasonal Variation

A. marmelos, *A. indica*, *C. fistula*, *Dalbergia sissoo*, and *T. bellirica* samples were collected across winter, summer, and rainy seasons to assess the impact of seasonal variations on metabolite synthesis and effect on their MS1 scores (Table S16). The effect of environmental conditions or metabolite synthesis

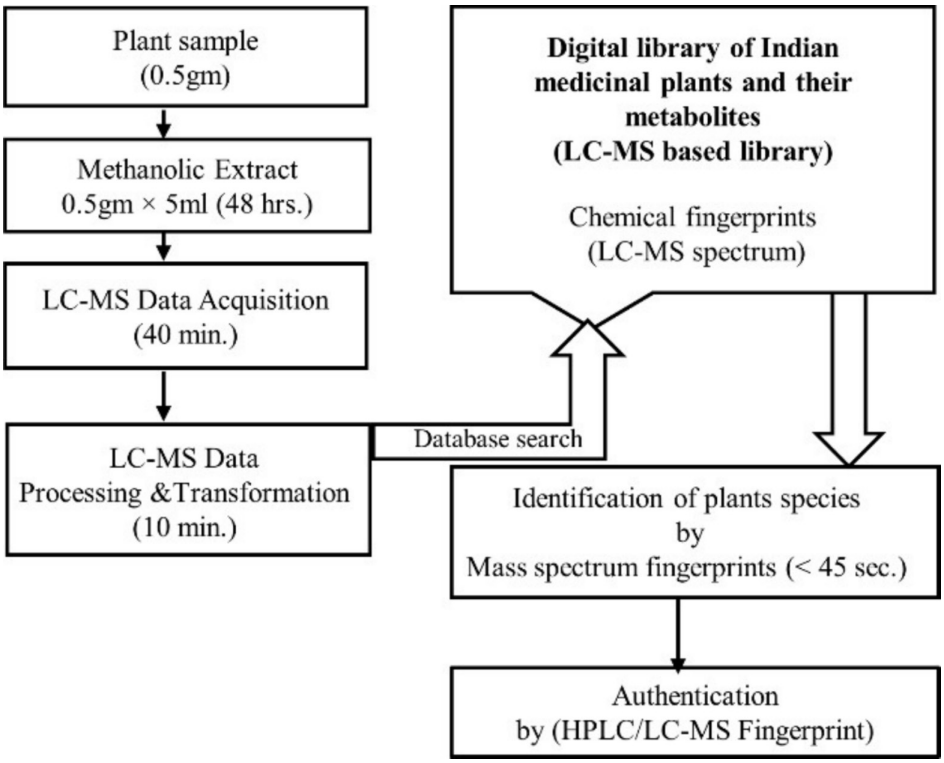

FIGURE 2 | Flow diagram for botanicals identification and authentication based on LC–MS fingerprints.

TABLE 1 | Identified plant species based on MSFP and their MS1 score.

S. no	Plant name	Accuracy	Reproducibility ($n = 3$)	ESI (±) average	
				MS1[a] score	Hit
1.	*Aegle marmelos*	100%	100%	60.86 ± 0.75	46
2.	*Amomum subulatum*	100%	100%	62.23 ± 0.66	47
3.	*Andrographis paniculata*	100%	100%	56.43 ± 9.33	42
4.	*Azadirachta indica*	100%	100%	55.56 ± 0.75	42
5.	*Bergera koenigii*	100%	100%	58.70 ± 0.00	44
6.	*Bombax ceiba*	100%	100%	53.00 ± 2.65	40
7.	*Butea monosperma*	100%	100%	56.86 ± 0.75	43
8.	*Cannabis sativa*	100%	100%	62.36 ± 0.75	47
9.	*Cassia fistula*	100%	100%	63.13 ± 3.37	47
10.	*Catharanthus roseus*	100%	100%	58.70 ± 0.00	44
11.	*Cinnamomum tamala*	100%	100%	64.43 ± 0.75	48
12.	*Cinnamomum verum*	100%	100%	59.86 ± 2.80	45
13.	*Dalbergis sissoo*	100%	100%	73.36 ± 4.61	55
14.	*Eclipta prostrata*	100%	100%	53.76 ± 2.04	40
15.	*Elettaria cardamomum*	100%	100%	60.90 ± 2.02	46
16.	*Glycyrrhiza glabra*	100%	100%	75.46 ± 0.92	57
17.	*Justicia adhatoda*	100%	100%	75.10 ± 2.02	56
18.	*Lawsonia inermis*	100%	100%	89.76 ± 4.08	67
19.	*Mangifera indica*	100%	100%	71.13 ± 0.75	53
20.	*Mentha suaveolens*	100%	100%	59.10 ± 5.36	44
21.	*Phyllanthus emblica*	100%	100%	68.90 ± 2.76	52
22.	*Rauvolfia serpentina*	100%	100%	73.33 ± 3.49	55
23.	*Syzygium aromaticum*	100%	100%	77.33 ± 1.35	58
24.	*Syzygium cumini*	100%	100%	68.43 ± 0.75	51
25.	*Terminalia arjuna*	100%	100%	61.33 ± 7.03	46
26.	*Terminalia bellirica*	100%	100%	64.00 ± 1.30	48
27.	*Terminalia chebula*	100%	100%	71.13 ± 0.75	53
28.	*Tinospora cordifolia*	100%	100%	52.33 ± 0.75	39
29.	*Trachyspermum ammi*	100%	100%	60.43 ± 0.75	45
30.	*Zingiber officinale*	100%	100%	74.20 ± 1.55	56

Note: ESI (±) = electrospray ionization positive and negative ionization mode, Hit = matched *m/z*.
[a]MS1 score in % with their standard deviation.

during different seasons resulted in a variation of the MS1 score from SD ± 2.10 to ± 13.82.

3.10 | Effect of Geographical Variation

To examine the impact of geographic variations on MS1 scores, the test samples of *T. chebula* and *Bergera koenigii* were collected from different parts of the country (Table S17). The MS1 score results were shown as SD ± 6.0 in the case of the two tested samples.

3.11 | Accuracy and Selectivity

The analytical method provided the desired results for identifying 30 plant species, with a notable 100% accuracy and selectivity. No other plant species ranked within the top 3 search results

(MS1 scores > 50%). It is emphasized that choosing more than the top three MS1 scores could compromise accuracy due to the similar MSFP in the database (MS1 score < 50%). Besides this, selectivity was 100% against the presence of more than 1564 (ESI ±) MSFPs available in the database.

3.12 | Chemical Barcode

It was based on the molecular weight sequence of exclusive plant secondary metabolites that should be specific to a plant species, as molecular weights of carbazole alkaloids are present in *Bergera koenigii* (279, 293, 309, 323, 331, 347 Da) and metabolites of *D. sissoo* (202, 187, 390, 564, 464, 448, 610, 580, 710, 578 Da). Unique molecular weight sequence (UMWS, or chemical barcode) analysis follows an SOP for botanical identification as shown in Figure 4. In the analysis, it has been observed that each species possesses a unique array of chemical compounds, herein referred to as a chemical barcode. The acquired LC–MS data were processed, and the molecular weights of 15–20 major metabolites for each plant species were determined and searched with the library. The search process involved entering the metabolite's molecular weights in their elution order separated by a comma into the 'molecular weight search' option of the library. The search results of the analysis are shown in Table S18. Thus, the UMWS, or chemical

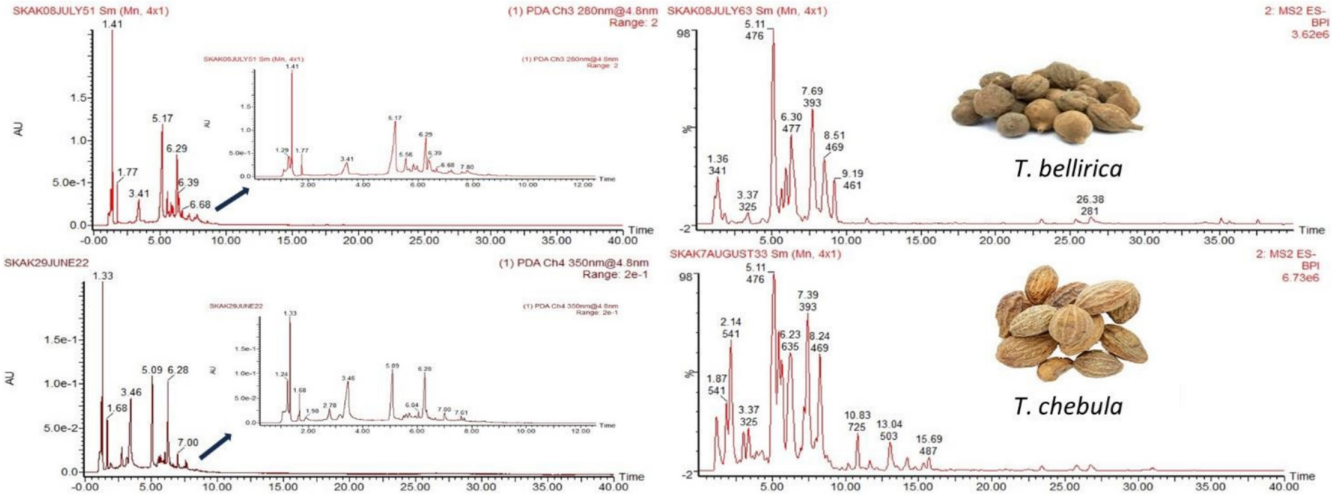

FIGURE 3 | UHPLC and MS chromatogram of *Terminalia bellirica* and *Terminalia chebula*.

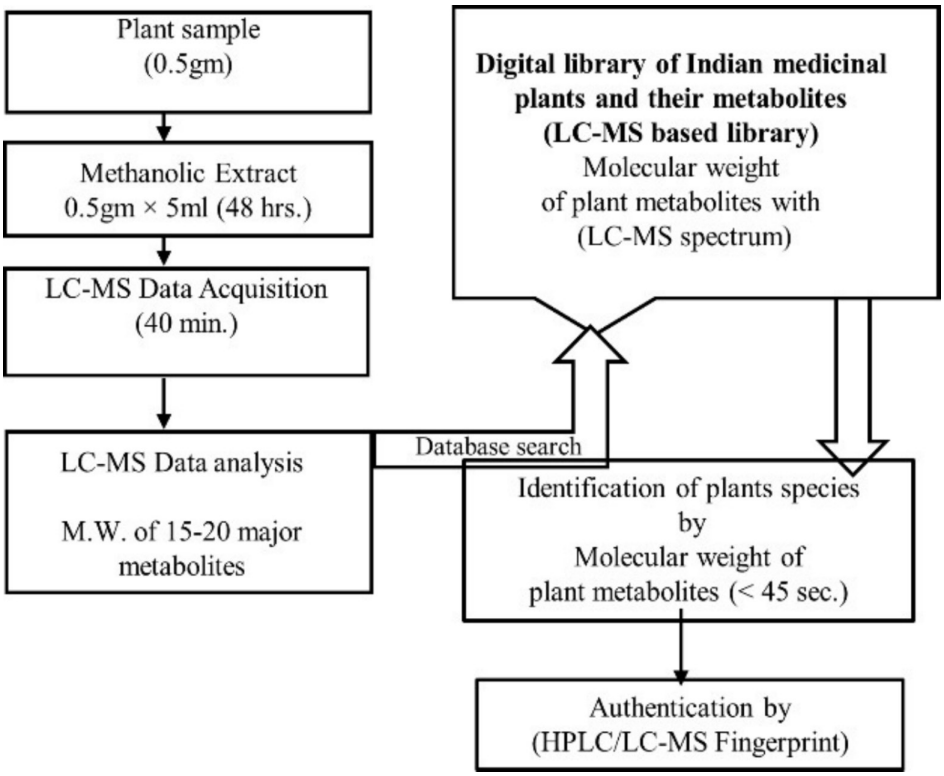

FIGURE 4 | Flow diagram for botanicals identification and authentication based on unique molecular weight sequence.

barcode approach, facilitates wider applicability, allowing diverse LC–MS instruments to be utilized for the identification of botanicals.

4 | Conclusion

We successfully tested 30 diversified botanical samples for their authenticity based on MSFP, and the results were consistently affirmative. Simultaneously, the multilevel validation of SOP established the accuracy, selectivity, and reproducibility of the results. Despite these strengths, certain limitations remain, such as the requirement for sophisticated instrumentation and the continuous expansion of reference libraries to ensure comprehensive coverage of diverse plant species. However, it has also been demonstrated that the chemical barcode can get beyond particular instrumental biases in botanical identification. MSFP offers significant advantages over the classical morphotaxonomy, microscopic pharmacognosy, or DNA barcoding methods. Not only does it ensure the chemical integrity but also makes it a valuable asset for detecting adulteration, ensuring batch-to-batch consistency and ensuring quality herbal raw material. Consequently, the LC–MS as an analytical tool and MSFP reference library may become the foundation of botanical identification with their chemical integrity.

Author Contributions

Sanjeev Kanojiya: conceptualization, experiment design, observation, suggestions, manuscript modifications. **Akhilesh Kumar:** sample collection, sample preparation, mass spectral data acquisition, observation, data processing, data analysis, and manuscript preparation. **Dipak K. Mishra:** plants raw material authentication for the development of a mass spectrum fingerprints reference library, review of the manuscript, and corrections.

Acknowledgments

Akhilesh Kumar gratefully acknowledges Mr. Shiv Nandan, Mr. Piush Srivastava, Ms. Nisha, Dr. Sumit Kumar Singh, and Er. Santosh Kumar Shukla for their contribution to the development of the digital library of Indian medicinal plants and their metabolites. The authors acknowledge the University Grant Commission and Council of Scientific Industrial Research, New Delhi, for the financial support in the form of a Junior Research Fellow (JRF) as well as a Senior Research Fellowship (SRF). The authors further acknowledge the Sophisticated Analytical Instrument Facility (SAIF Lucknow), where LC–MS studies were carried out. The authors are grateful to the project (YSS/2015/001048) funded by the Science & Engineering Research Board (SERB) DST Government of India for financial support for the development of a digital library. CSIR-CDRI communication number is 10952.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data analysis was performed using the plant metabolites database (https://plantmetabolome.cdri.res.in). It is freely accessible and utilized as a reference library. All the results related to this work are included in the supporting information. The input data files (*.txt or *. tmsd) that support the findings of this study are available on reasonable request to the corresponding author.

References

1. J. M. Rajput, D. S. Nandre, and B. G. Pawar, "A Comprehensive Review on Advanced Chromatographic Techniques and Spectroscopic Techniques in Pharmaceutical Analysis," *International Journal of Pharmaceutical Research and Applications* 7, no. 3 (2022): 53–62.

2. N. Kumar, A. Bansal, G. Sarma, and R. K. J. T. Rawal, "Chemometrics Tools Used in Analytical Chemistry: An Overview," *Talanta* 123 (2014): 186–199.

3. M. A. Farag, M. Sheashea, C. Zhao, and A. A. Maamoun, "UV Fingerprinting Approaches for Quality Control Analyses of Food and Functional Food Coupled to Chemometrics: A Comprehensive Analysis of Novel Trends and Applications," *Food* 11, no. 18 (2022): 2867.

4. R. Kumar and V. Sharma, "Chemometrics in Forensic Science," *TrAC Trends in Analytical Chemistry* 105 (2018): 191–201.

5. H. L. Pennington and S. A. Weber, "Paleoethnobotany: Modern Research Connecting Ancient Plants and Ancient Peoples," *Critical Reviews in Plant Sciences* 23, no. 1 (2004): 13–20.

6. R. L. Usinger, "The Role of Linnaeus in the Advancement of Entomology," *Annual Review of Entomology* 9, no. 1 (1964): 1–17.

7. N. Kumar, P. N. Belhumeur, A. Biswas, et al., "Computer Vision–ECCV 2012. Lecture Notes in Computer Science," (2012), 502–516.

8. J. S. Cope, D. Corney, J. Y. Clark, P. Remagnino, and P. Wilkin, "Plant Species Identification Using Digital Morphometrics: A Review," *Expert Systems with Applications* 39, no. 8 (2012): 7562–7573.

9. N. J. Haider, "Identification of Plant Species Using Traditional and Molecular-Based Methods," in *Wild Plants: Identification, Uses and Conservation*, ed. R. E. Davis (Nova Science Publishers, Inc., 2011): 1–62.

10. H. C. Wittich, M. Seeland, J. Wäldchen, M. Rzanny, and P. J. Mäder, "Recommending Plant Taxa for Supporting on-Site Species Identification," *BMC Bioinformatics* 19 (2018): 1–17.

11. S. K. Pal and Y. Shukla, "Herbal Medicine: Current Status and the Future," *Asian Pacific Journal of Cancer Prevention* 4, no. 4 (2003): 281–288.

12. P. J. Wangchuk, "Therapeutic Applications of Natural Products in Herbal Medicines, Biodiscovery Programs, and Biomedicine," *Journal of Biologically Active Products from Nature* 8, no. 1 (2018): 1–20.

13. A. C. Raclariu, M. Heinrich, M. C. Ichim, and H. de Boer, "Benefits and Limitations of DNA Barcoding and Metabarcoding in Herbal Product Authentication," *Phytochemical Analysis* 29, no. 2 (2018): 123–128.

14. P. Mishra, A. Kumar, A. Nagireddy, et al., "DNA Barcoding: An Efficient Tool to Overcome Authentication Challenges in the Herbal Market," *Plant Biotechnology Journal* 14, no. 1 (2016): 8–21.

15. H. A. Gad, S. H. El-Ahmady, M. I. Abou-Shoer, and M. M. Al-Azizi, "Application of Chemometrics in Authentication of Herbal Medicines: A Review," *Phytochemical Analysis* 24, no. 1 (2013): 1–24.

16. L. Tnah, S. Lee, A. Tan, et al., "DNA Barcode Database of Common Herbal Plants in the Tropics: A Resource for Herbal Product Authentication," *Food Control* 95 (2019): 318–326.

17. W. L. Applequist, J. S. Miller, and B. Chemistry, "Selection and Authentication of Botanical Materials for the Development of Analytical Methods," *Analytical and Bioanalytical Chemistry* 405 (2013): 4419–4428.

18. S. H. Ganie, P. Upadhyay, S. Das, and M. P. Sharma, "Authentication of Medicinal Plants by DNA Markers," *Plant Gene* 4 (2015): 83–99.

19. D. Hao, S. Chen, and P. Xiao, "Authentication of Medicinal Plants Based on Molecular Biology and Genomics," *Chinese Journal of Pharmaceutical Biotechnology* 16 (2009): 490–494.

20. B. Mohammed Abubakar, F. Mohd Salleh, M. S. Shamsir Omar, and A. Wagiran, "DNA Barcoding and Chromatography Fingerprints

for the Authentication of Botanicals in Herbal Medicinal Products," *Evidence-based Complementary and Alternative Medicine* 2017, no. 1 (2017): 1352948.

21. T. Smillie and I. Khan, "A Comprehensive Approach to Identifying and Authenticating Botanical Products," *Clinical Pharmacology & Therapeutics* 87, no. 2 (2010): 175–186.

22. S. Sultana, M. A. Khan, M. Ahmad, A. Bano, M. Zafar, and Z. K. Shinwari, "Authentication of Herbal Medicine Neem (Azadirachta Indica a. Juss.) by Using Taxonomic and Pharmacognostic Techniques," *Pakistan Journal of Botany* 43 (2011): 141–150.

23. N. Techen, I. Parveen, Z. Pan, and I. A. Khan, "DNA Barcoding of Medicinal Plant Material for Identification," *Current Opinion in Biotechnology* 25 (2014): 103–110.

24. C. Liu, Z. Zuo, F. Xu, and Y. Wang, "Authentication of Herbal Medicines Based on Modern Analytical Technology Combined With Chemometrics Approach: A Review," *Critical Reviews in Analytical Chemistry* 53, no. 7 (2023): 1393–1418.

25. K. Vijayan and C. Tsou, "DNA Barcoding in Plants: Taxonomy in a new Perspective," *Current Science* 99, no. 11 (2010): 1530–1541.

26. N. Techen, S. Crockett, I. Khan, and B. J. Scheffler, "Authentication of Medicinal Plants Using Molecular Biology Techniques to Compliment Conventional Methods," *Current Medicinal Chemistry* 11, no. 11 (2004): 1391–1401.

27. D. de Carvalho Lopes and A. J. S. Neto, "Classification and authentication of plants by chemometric analysis of spectral data," *Comprehensive Analytical Chemistry* 80. Elsevier (2018): 105–125.

28. W. Gao, H. Yang, L.-W. Qi, et al., "Unbiased Metabolite Profiling by Liquid Chromatography–Quadrupole Time-Of-Flight Mass Spectrometry and Multivariate Data Analysis for Herbal Authentication: Classification of Seven Lonicera Species Flower Buds," *Journal of Chromatography A* 1245 (2012): 109–116.

29. D. Ved and G. J. N. Goraya, "Demand and Supply of Medicinal Plants in India," *NMPB, New Delhi & FRLHT, Bangalore, India* 18, no. 85 (2007): 210–252.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.