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RESEARCH ARTICLE

Identification of Botanicals Based on Their Chemical Barcode Using Ultraperformance Liquid Chromatography–Mass Spectrometry

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ABSTRACT

Rationale: Plants synthesize diverse secondary metabolites, often specific to particular species. These metabolites (phytochemicals) exhibit restricted distribution among certain plant families, genera, or species. Due to their species-specific characteristics, they can serve as chemical markers to identify and authenticate economically important botanicals.

Method: Ultrahigh performance liquid chromatography–mass spectrometry (UHPLC–MS) and a plant metabolome reference library were utilized to identify and authenticate botanicals based on their characteristic phytochemicals.

Result: A chemical barcode refers to a unique molecular weight sequence (e.g., M.W. 216, 392, 390, 438, 542, 368, and 495 for *Curcuma longa*) of characteristic phytochemicals of particular plant species. This study reports the utilization of a plant metabolome library, LC–MS, and MS/MS data to facilitate the identification of botanicals based on their chemical barcode. We have analyzed 20 economically important medicinal plant species (*A. nilotica*, *A. calamus*, *A. scholaris*, *B. monnieri*, *B. diffusa*, *C. asiatica*, *C. sativus*, *C. longa*, *F. religiosa*, *M. alba*, *M. fragrans*, *N. sativa*, *O. tenuiflorum*, *P. amarus*, *P. betel*, *P. longum*, *P. nigrum*, *P. pinnata*, *S. asoca*, and *V. negundo*); no false results were observed in multiple tested samples. Apart from this, barcoded metabolites were also identified to validate the results based on previously reported phytochemicals from respective plant species and their mass spectrometry data.

Conclusion: This approach facilitated the successful identification of botanicals through their chemical barcode and utilized a web-based LC–MS/MS library of nontargeted plant secondary metabolites.

1 | Introduction

Plants synthesize diversified organic compounds or metabolites [1]; some play crucial roles in various fundamental biological processes, like photosynthesis, growth, respiration, reproduction, and storage [2]. These metabolites are known as primary metabolites, whereas phytochemicals with no role in primary metabolism are called secondary metabolites [3]. They often display restricted distribution among particular plant families, genera, or species. This restricted occurrence reflects the evolutionary

Abbreviations: Hr, hour; MRM, multiple reaction monitoring; *m/z*, mass-to-charge ratios; RT, retention time; SOP, standard operating procedure; Th, Thomson; UHPLC-ESI-MS/MS, ultrahigh performance liquid chromatography–tandem mass spectrometer; UMWS, unique molecular weight sequence.

adaptations and ecological roles associated with secondary metabolites, which have emerged through specialized biosynthetic pathways in distinct lineages of plants [4]. These metabolites are specific to particular plant species and serve as chemical markers for those plants. For example, *Azadirachta indica* (neem) contains limonoids [5], like nimbin, nimbidin, and nimbolide; *Bergera koengii* (curry leaf) contains carbazole alkaloids like mahanimbine, girinimbine, koenimbine, isomahanine, and mahanine [6]; and *Terminalia chebula* (harad) synthesizes chebulic acid, chebulanin, terchebulanin, chebulagic, and neochebulic acid as its unique secondary metabolites [7]. The unique distribution patterns of secondary metabolites contribute to their significance in plant taxonomy, phylogenetics, and ecological interactions within natural ecosystems [8]. Due to their specific and limited distribution, secondary metabolites can serve as valuable diagnostic constituents in chemotaxonomic studies [9]. These unique secondary metabolites offer numerous potential applications that could benefit various sectors. Their specific chemical structures and biological activities make them attractive candidates for drug discovery, as they may possess therapeutic properties against various diseases [10]. Moreover, the chemotaxonomic significance of secondary metabolites could aid in identifying and authenticating plant species, contributing to the preservation of traditional knowledge associated with medicinal plants [11].Additionally, in plant metabolome analyses, tandem mass spectrometry (MS/MS) emerges as a potent analytical technique for the identification of chemical compounds or phytochemicals [12]. During MS/MS, metabolites undergo fragmentation upon collision with a collision gas (such as helium), generating a fragmentation pattern captured in a product ion spectrum [13]. This spectrum records the *m/z* values of product ions and their relative intensities, forming unique fingerprints that aid in metabolite identification, particularly for distinguishing structurally isomeric metabolites [14]. Hence, the phytochemical signature of secondary metabolites, along with their characteristic MS/MS spectral patterns, can be effectively utilized for the identification of plant species. Traditionally, plants have been identified using several methods, like morphotaxonomy and DNA barcoding [15–17]. However, these methods have certain limitations. Morphotaxonomy relies on physical characteristics, which can be influenced by human interpretation, leading to potential misidentification. On the other hand, DNA barcoding is a molecular approach that depends on the integrity of genetic material, making it less effective when samples are degraded or processed [18–20] Herein, we introduce chemical barcode as a unique molecular weight sequence, which is uniquely tailored for each plant species through comprehensive LC–MS data analysis. This chemical barcode is employed as the initial stage for the identification of botanicals based on their characteristic phytochemicals. Further, to enhance the reliability of results, each unique metabolite's MS/MS fingerprint is used for authentication. Thus, LC–MS and LC–MS/MS play a pivotal role in the analysis of phytochemicals to advance the authentication of economically important botanicals. In this work, an open-access plant metabolome library [21] is used for the chemical barcode and MS/MS fingerprint search of specific markers for individual plant species. The utilized plant metabolome library has more than 300 plant species from 104 plant families, incorporating more than 4800 MS/MS spectra of plant metabolites. Thus, this approach facilitates accurate and efficient identification and authentication of botanicals, providing valuable support for herbal raw material authentication and quality control in botanical products.

2 | Experimental

2.1 | Chemicals and Materials

Analytical-grade (LC–MS) acetonitrile (CH_3CN) and ammonium acetate ($NH_4CH_3CO_2$) were purchased from Merck Life Science (Mumbai, India) and Finar (Gujarat, India), respectively. At the same time, HPLC-grade methanol (CH_3OH) was purchased from Molychem (Mumbai, India). Deionized triple-distilled water was prepared using a Milli-Q water system (Merck Millipore, Bangalore, India).

2.2 | Selection of Plant Materials

This study analyses high-value medicinal plants native to India. These plants hold immense importance within the nation's healthcare and herbal sectors, serving as key ingredients in traditional remedies, pharmaceuticals, and herbal products.

2.3 | Sample Collection and Preparation

Plant materials were collected from the Lucknow region (26.8467° N, 80.9462° E) in the upper Gangetic plains, with additional samples purchased from the local market for LC–MS analysis. Authentication of plant materials was carried out by a coauthor and angiosperm taxonomist (DKM). A detailed description of the plant samples is provided in Table S1. The collected plant materials were dried in a conventional oven at 35°C–37°C, ground into a coarse powder, and stored in sealed vials at room temperature. For extraction, 500 mg of each powdered sample was mixed with 5 mL of CH_3OH and sonicated in an ultrasonic water bath for 5 min after an initial incubation of 24 h. The mixture was then allowed to stand for 48 h at room temperature (~25°C). The resulting methanolic extracts were filtered through a 0.2 μm syringe filter and transferred into sample vials for LC–MS analysis.

2.4 | Data Acquisition

The hyphenated UHPLC-PDA-ESI-MS/MS system, developed by Waters Co., Milford, Massachusetts, United States, combines ultrahigh-performance liquid chromatography (UHPLC) with photodiode array (PDA) detection and a Waters Xevo TQD mass spectrometer interface utilizing electrospray ionization (ESI). This hybrid system facilitates the simultaneous acquisition of UHPLC and MS profiles. The chromatographic separation was achieved using a mobile phase composed of (A) CH_3CN and (B) 5 mM $NH_4CH_3CO_2$ buffer, which was passed through a Waters BEH C-18 column (150 × 2.1 mm, 1.7 μm) at a constant flow rate of 0.250 mL/min, employing a linear gradient shown in Table S2. The UV-PDA spectra were acquired from 200 to 450 nm, while fullscan mass spectrometric data were acquired in both ESI-positive and ESI-negative ion modes, covering a mass range from *m/z* 150 to 1500 Th. MS/MS experiments were also conducted in both ESI-positive and ESI-negative ion modes.

ESI parameters were maintained at a capillary voltage of 3.5 kV and a cone voltage of 30 V. Nitrogen served as the nebulizing and drying gas, with flow rates set at 50 and 950 L/h, respectively. The source and desolvation temperatures were set at 120 and 350 °C, respectively, and argon gas was utilized for collision-induced dissociation (CID). Data acquisition and processing were facilitated using MassLynx V4.1 software.

2.5 | Data Interpretation and Transformation

The molecular weights of plant metabolites were determined based on observed adduct ions in the ESI spectra as illustrated in Figure S1. Subsequently, molecular weight assignment, a unique molecular weight sequence was created for each plant species by selecting major metabolites that were distinct to a particular plant species based on multiple searches in the plant metabolome library and consistently detected across tested samples. This sequence served as a chemical barcode, enabling species differentiation regardless of seasonal and geographical metabolic variations. Further, MS/MS data transformation was performed using MassLynx V4.1. The comprehensive workflow for the conversion of MS/MS spectra into text format is provided in the supplementary file in Figures S2 and S3.

2.6 | Library Search

A pictorial screenshot of the library search is visually represented in Figures S4 and S5. The search process began by accessing the plant metabolome library (https://plantmetabolome.cdri.res.in) and completing the registration process. Upon successful verification via email, the assigned login credentials were used to access the mass spectrometry-based search section. For chemical barcode searches, the molecular weight option was selected, and the molecular weight was entered, separated by commas, or a Notepad file containing the chemical barcode data was uploaded directly. Once the search was executed, the results were displayed on screen, as illustrated in Figure S4. Similarly, for the MS/MS library search, the corresponding screenshot is provided in Figure S5.

3 | Results and Discussion

3.1 | Reference Library

This reference library outlines a standard operating procedure (SOP) for the identification of plant species using chemical barcode and MS/MS chemical fingerprints of phytochemicals. The protocol covers the entire workflow, from sample preparation to library search, ensuring a systematic approach to botanical identification. In this study, 0.5 g of plant material was extracted with 5 mL of CH_3OH for 48 h. The resulting extract was analyzed using a triple quadrupole mass spectrometer coupled with a UHPLC system. The acquired LC–MS data facilitated determining the molecular weights of 15–20 major metabolites, which were used for search in the library as chemical barcodes. Further, validation of results was performed using MS/MS fragments and chromatographic elution order of these key metabolites (barcoded metabolites) and confirming their identity as chemical markers. The pictorial overview from sample preparation to library search for chemical barcode as well as MS/MS fingerprints is shown in Figure 1.

3.2 | Identification of Plant Species by Chemical Barcode

Initially, the selected botanicals were identified using a chemical barcode, which refers to a unique sequence of molecular weights corresponding to specific phytochemicals present in a given plant species. The unique molecular weight sequence of the phytochemicals was assigned based on their elution order in the chromatographic run, considering only nonrepeated values. This sequence served as a chemical barcode for searching in the reference library. In the analysis of distinct plant species, it has been observed that each species possesses a unique array of chemical compounds, herein referred to as chemical markers. These chemical markers act as distinctive identifiers (chemical barcode) for each plant species like for *Curcuma longa* (M.W. 216, 392, 390, 438, 542, 368, 495), *Acacia nilotica* (M.W. 594, 329, 564, 315, 313, and 299) *Centella asiatica* (M.W. 504, 354, 516, 462, 975, 959, and 519), and *Piper*

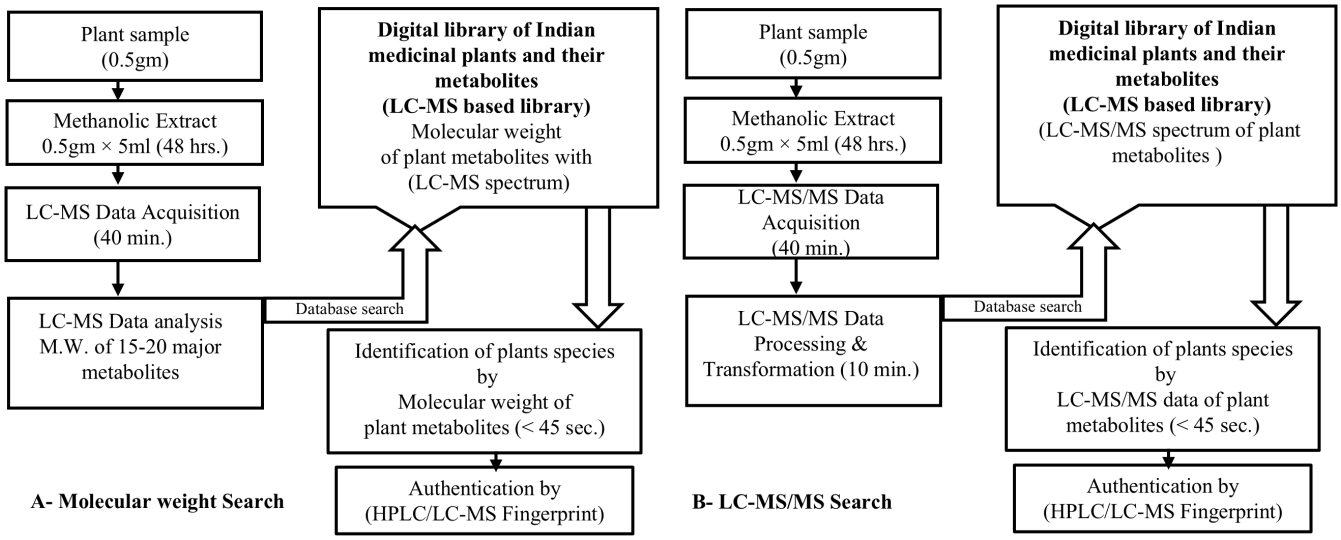

FIGURE 1 | SOP for molecular weights (chemical barcode) and LC-MS/MS fragments search of chemical markers.

nigrum (M.W. 261, 257, 327, 329, 341, 235, and 343). As a result of the reference library search from the chemical barcode of unknown plant species, a list of plant species and their corresponding chemical barcodes matched total hits is presented in Table 1. Further, the chemical barcode sequence was further validated by the chromatographic retention time (± 0.8 min) of the identified metabolites. In addition to this, evaluated other closely matched plant species to verify the uniqueness of constructed chemical barcode and data is reported in Table S3. The second and third closest matched chemical barcodes were further verified by chromatographic retention time (± 0.8 min) of the hit metabolites. But the elution order and retention time was mismatched. Thus, it confirmed the uniqueness of identifiers (chemical barcode).

3.2.1 | Effect of Isobaric Compounds

In this study, the uniqueness of chemical barcodes was crucial for the accurate identification of botanicals. However, the presence of common compounds, such as organic acids, sugars, flavonoids, and fatty acids like palmitic acid, stearic acid, oleic acid, and linoleic acid with similar molecular weights posed significant challenges to achieving selectivity in the chemical barcode. These compounds' molecular weights increased the number of hits, while reducing selectivity and acting as interfering agents for the uniqueness of identifiers (chemical barcode). To address this issue, we need to exclude these interfering molecular weights through chromatographic data validation and enhanced selectivity of botanical identification.

TABLE 1 | Identification of plant species by chemical barcode.

Molecular weights of plant metabolites based on their elution order		Search result of chemical barcode from library (https://plantmetabolome.cdri.res.in/)				
S. no.	**Chemical barcodes[a]**	**Plant Name**	**No. of metabolites[$]**	**Total metabolites[@]**	**Total hits[#]**	**Selectivity (%)**
1.	594,329,564,315,313,299	*Acacia nilotica*	6	37	15	100
2.	196,224,222,234,210,208,220	*Acorus calamus*	7	30	13	100
3.	340,385,352,338,366,386	*Alstonia scholaris*	6	35	10	100
4.	478,1014,652,270,928,454,898,506	*Bacopa monnieri*	8	31	09	100
5.	624,330,654,492,314,32 8,342,298,312,326	*Boerhavia diffusa*	10	24	16	100
6.	504,354,516,462,975,959,519	*Centella asiatica*	7	19	10	100
7.	316,330,448,652,328,814,669,519	*Crocus sativus*	8	41	16	100
8.	216,392,390,438,542,368,495	*Curcuma longa*	7	23	13	100
9.	396,426,236,201,370,495	*Ficus religiosa*	6	54	10	100
10.	178,626,216,386,610,464,648	*Morus alba*	7	39	07	100
11.	326,344,208,374,356,370,324	*Myristica fragrans*	7	28	16	100
12.	204,286,772,304,164, 454,750,676,222	*Nigella sativa*	9	39	18	100
13.	226,360,462,312,313,444,506	*Ocimum tenuiflorum*	7	20	08	100
14.	221,276,260,418,354, 386,400,432,608	*Phyllanthus amarus*	9	32	12	100
15.	192,176,150,174,234,165,206	*Piper betel*	7	12	09	100
16.	265,316,150,222,367,208,220,273	*Piper longum*	8	84	13	100
17.	261,257,327,329,341,235,343	*Piper nigrum*	7	20	07	100
18.	314,396,352,410,336, 326,366,380,350	*Pongamia pinnata*	9	41	16	100
19.	578,290,448,432,463,268,304,519	*Saraca asoca*	8	32	12	100
20.	496,466,360,346,374, 388,377,270,272	*Vitex negundo*	9	40	11	100

[a]Unique molecular weight sequence of metabolites.
$Total number of metabolites in chemical barcode.
@Total metabolites in the library against respective plant species.
#Number of metabolite's molecular weight matched.

3.2.2 | Constraint of Chemical Barcode

The chemical barcode approach has certain limitations; metabolites (phytochemicals) serve as chemical markers. It must be a major constituent with consistent availability in different sessions and geographical conditions. However, the expression levels of metabolites may vary due to seasonal and geographical or environmental changes. Over a period of time, the chemical barcode can also be modified due to the expression of additional metabolites (chemical markers) and potential improvements in botanical identification practices. Ultimately, the uniqueness of chemical barcodes plays a crucial role in the selective identification of botanicals.

3.3 | Identification of Plant Species by Their Chemical Markers

Selection of chemical markers is crucial for the quality control of plant-based medicines, including the authentication of genuine species. The discovery of chemical markers for the identification of plant species is one of the major challenges in herbal products. The pharmacopeia defines a "chemical marker" as a distinct chemical component found in a plant or herbal medicine that is used to identify and assess the material's quality [22]. It allows for consistent quality control from the harvesting of raw materials to drug production. Thus, a SOP was rigorously followed to ensure consistency and reproducibility in the results. For the authentication of botanical raw materials, a SOP includes LC–MS/MS data acquisition, transformation, and library search for chemical markers or barcoded metabolites. During the chemical barcode search from the reference library, molecular weight sequences exposed similarities due to isobaric metabolite matching like *Acorus calamus*, *Piper longum*, and *Cassia fistula*, as depicted in Table S3. To address this challenge, the Top 5 molecular fragments were also taken into consideration (as shown in Table 2) for the library search. The MS/MS fragments of selected chemical markers were successfully utilized to identify previously known compounds from evaluated plant species. It provided three-stage selectivity based on chromatographic retention time, molecular weight, and MS/MS fragments of chemical markers. For the validation of the current methodology, 20 different plant species were evaluated using targeted MS/MS fragmentation of chemical markers; they were clearly distinguished from one another by comparing their characteristic chemical profiles. It can be revealed that *A. calamus* has β-asarone, *Alstonia scholaris* contained echitamidine, *Boerhavia diffusa* contained boeravinone D, *Crocus sativus* contained picrocrocin, and *P. longum* contained piperlonguminine (Table 2). Apart from this, some selected plants' chemical markers were identified based on previous literature and mass spectrometry data for validation of results.

3.3.1 | *A. scholaris*

Based on LC–MS/MS analysis, the chemical profiling of *A. scholaris* revealed the presence of multiple metabolites, each characterized by distinct retention times and molecular weights in positive ionization mode. Key identified metabolites included echitamidine (Rt 3.34 min, M.W. 341), 3-epi-dihydro corymine-17-acetate (Rt 9.72 min, M.W. 385), and scholarisine (Rt 10.62 min, M.W. 387). Additionally, compounds such as picrinine (Rt 13.42 min, M.W. 353), dehydrated scholaricine (Rt 15.38 min, M.W. 339), and picralinal (Rt 15.64 min, M.W. 367) were also detected (Table 2). The identification of these metabolites was further supported by their characteristic fragmentation patterns, yielding diagnostic product ions at *m/z* 270, 220, 200, 170, 107, and 106, which corroborated their structural assignments.

3.3.2 | *B. diffusa*

The chemical characterization of compounds extracted from *B. diffusa* was identified. Metabolites include eupalitin, eupalitin 3-galactoside, boeravinones (A, B, D, and E), and 9-hydroxy-2-octadecenoic acid, with their respective Rt, *m/z*, and characteristic fragment ions documented. Additionally, several compounds remain unidentified, including those detected at Rt 7.99 min (*m/z* 625), 9.87 min (*m/z* 655), and 12.65 min (*m/z* 315) (Table 2). The fragmentation patterns, presented with relative abundances, facilitated compound identification through spectral library matching and comparison with previously published literature. This analysis underscores the phytochemical diversity of *B. diffusa*, with potential implications in drug discovery, chemotaxonomy, and the characterization of its bioactive constituent.

3.3.3 | *Myristica fragrans*

The characterization of compounds extracted from *M. fragrans* includes nectandrin-B (Rt 15.86 min, *m/z* 343), licarin A (Rt 15.93 min, *m/z* 327), eemecin (Rt 16.25 min, *m/z* 209), and fragransols (A, C, D) along with licarin B. Each compound is described by unique fragmentation patterns with relative intensities, such as nectandrin-B showing 151(100), licarin A at 151(100), and fragransol A at 179(100). Fragment ions with dominant peaks provide molecular fingerprinting, aiding compound identification (Table 2). This characterization demonstrates the chemical diversity of *M. fragrans*, highlighting its potential utility in pharmaceutical, nutraceutical, and flavor applications.

3.3.4 | *Pongamia pinnata*

The Table 2 provides a mass spectrometric characterization of notable identified compounds including ponganone-VII (Rt 11.57 min, *m/z* 315), pongamipinone B (Rt 14.66 min, *m/z* 397), and pongagallone A (Rt 15.12 min, *m/z* 353). Additional compounds, such as pongapinone (Rt 15.84, *m/z* 411), pongapin or gamatin (Rt 15.94 min, *m/z* 337), and demethoxykanugin (Rt 16.25 min, *m/z* 327), demonstrate the chemical complexity of this species (Table 2). Fragmentation patterns, such as dominant peaks at 297(100) for ponganone-VII and 322(100) for pongapin, aid in precise molecular identification. The dataset underscores the phytochemical diversity and potential bioactivity of *P. pinnata*, offering insights into its possible applications in pharmacology and natural product research.

TABLE 2 | List of identified plant species using plant metabolome library considering Top 5 MS/MS fragments of chemical markers.

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
1.	*Acacia nilotica*	594,329,564,315,313,299	5.04	595	Catechin-7,3 -di-O-gallate [24]	Level 2b	595 (100), 596 (29), **577(66)**, 475(19), 457(40),	1.0	$[M+H]^+$	100
			5.70	330	Vanillic acid-O-hexoside [24]	Level 2b	330(39), 312(40), **294(100),** 81(14), 70(40)	1.0	$[M+H]^+$	
			5.91	563	Not identified	Level 5	384(11), 383(62), 365(10), 354 (19), **353(100)**	1.0	$[M-H]^-$	
			8.86	316	Not identified	Level 5	316(27), 298(40), 281(20), **280 (100**), 70(57)	1.0	$[M+H]^+$	
			9.79	314	Not identified	Level 5	**296(100),** 278(23), 95(22), 81(22), 70(63)	1.0	$[M+H]^+$	
			13.72	300	Salicylic acid-O-hexoside [25]	Level 2b	**282(100)**, 95(25), 81(26), 70(66), 69(21)	1.0	[M+H]+	
2.	*Acorus calamus*	196,224,222,234,210,208,220	10.94	197	3,4,5-Trimethoxybenzaldehyde [26]	Level 2b	169(79), 167(30), 154(77), 139(36), **138(100**)	1.0	[M+H]+	100
			7.02	225	1-(2,4,5-Trimethoxyphenyl) propan-2-one [26]	Level 2b	193(74), 168(69), 167(67), **165(100)**, 162(37)	1.0	[M+H]+	
			13.09	223	3-(2,4,5-Trimethoxyphenyl)-2-propenal [26]	Level 2b	195(34), 192(33), **191(100),** 176(52), 55(40)	1.0	[M+H]+	
			15.35	235	Not identified	Level 5	235(46), 179(52), 161(66), 81(56), **69(100)**	1.0	[M+H]+	
			15.57	211	Linalyl propionate [26]	Level 2b	211(7), 196(2), 183(3), 168(10), **167(100)**	1.0	[M+H]+	
			15.91	209	Beta-Asarone [26]	Level 2b	209(41), 194(89), **181(100),** 179(80), 178(55)	1.0	[M+H]+	
			21.39	221	Shybunone, acorenone B [26]	Level 2b	153(12), **83(100**), 81(40), 71(12), 69(17)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
3.	*Alstonia scholaris*	340,385,386,352,338,366	3.34	341	Echitamidin [26]	Level 2b	**200(100),** 194(84), 184(82), 182(71), 172(82)	1.0	[M+H]+	100
			9.72	385	3-epi-dihydrocorymine17-acetate [26]	Level 2b	385(52), 232(63), 222(55), **220(100),** 194(57)	1.0	[M+]+	
			10.62	387	Scholarisine [26]	Level 2b	355(46), **271(100),** 270(8), 265(39), 257(11)	1.0	[M+H]+	
			13.42	353	Picrinine [26]	Level 2b	247(58), 232(42), 220(79), 202(46), **170(100)**	1.0	[M+H]+	
			15.38	339	Dehydrated scholaricine [26]	Level 2b	206(12), 120(16), 108(54), 107(66), **106(100)**	1.0	[M+H]+	
			15.64	367	Picralinal [26]	Level 2b	270(30), 252(23), 108(55), **107(100),** 106(91)	1.0	[M+H]+	
4.	*Bacopa monnieri*	478,1014,652,270,928,454,898,506	7.50	477	Not identified	Level 5	477(32), 179(18), 162(9), **161(100),** 135(8)	1.0	[M-H]—	100
			7.56	1015	Not identified	Level 5	299(21), 269(5), 214(7), 213(50), **107(100**)	1.0	$[M+H]^+$	
			10.63	651	Not identified	Level 5	475(7), 193(17), 176(11), **175(100),** 113(14)	1.0	$[M-H]^-$	
			12.64	271	Apigenin [27]	Level 2b	271(51), **153(100),** 119(45), 91(21), 67(43)	1.0	[M+H]+	
			14.38	929	Bacoside A3 [27]	Level 2b	930(31), 474(38), **473(100),** 456(19), 455(52)	1.0	[M+H]+	
			14.54	455	Ebelin lactone [26]	Level 2b	**123(100),** 121(58), 109(91), 95(75), 81(60)	1.0	[M+H]+	
			14.63	899	Bacopaside [26]	Level 2b	960(5), 958(30), 901(2), 900(12), **898(100)**	1.0	$[M+CH_3COO]^-$	
			18.20	507	Not identified	Level 5	507(32), 257(17), **256(100),** 239(14), 238(74)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
5.	*Boerhavia diffusa*	624,330,654,492,314,328,342,298,312,326	7.99	625	Not identified	Level 5	625(28), 479(26), 318(8), 147(6), **317(100)**	1.0	[M+H]+	100
			9.88	331	Eupalitin [28]	Level 2b	331(86), 316(34), 298(98), **270(100),** 242(57**)**	1.0	[M+H]+	
			9.87	655	Not identified	Level 5	655(12), 494(4), 493(13), 332(20), **331(100)**	1.0	[M+H]+	
			11.22	493	Eupalitin 3-galactoside [28]	Level 2b	494(7), 493(24), 333(2), 332(17), **331(100**)	1.0	[M+H]+	
			12.65	315	Not identified	Level 2b	315(30), 297(27), **273(100),** 269(25), 245(19)	1.0	[M+H]+	
			14.42	329	Boeravinone E [26]	Level 2b	329(89), 311(32), 297(48), 287(77), **177(100)**	1.0	[M+H]+	
			15.51	343	Boeravinone D [26]	Level 2b	343(48), 311(43), 283(30), 240(40), **177(100)**	1.0	[M+H]+	
			16.12	299	9-hydroxyoctadecenoic acid [29]	Level 2b	299 (100), 300(35), 284(19), **166(72),** 137(24)	1.0	[M+H]+	
			16.30	313	Boeravinone B [26]	Level 2b	313(42), **271(100),** 295(24), 267(31), 167(25)	1.0	[M+H]+	
			18.61	327	Boeravinone A [26]	Level 2b	327(39), 295(49), 267(56), 162(18), **161(100)**	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
6.	*Centella asiatica*	504,354,516,462,975,959,519	1.36	505	Madecassic acid [30]	Level 2b	487(5), 343(9), **325(100),** 163(47), 145(5)	1.0	[M+H]+	
			2.13	355	Cholorogenic acid [30]	Level 2b	356(1) 355(6), 164(3), **163(100),** 79(1)	1.0	[M+H]+	
			5.81	517	3,5-di-Caffeoylquinic acid [31]	Level 2b	517(6), 500(14), 499(48), 355(5), **163(100)**	1.0	[M+H]+	
			7.83	463	Not identified	Level 5	463(2), 288(9), **287(100),** 159(4), 113(3)	1.0	[M+H]+	
			11.80	974	Madecassoside [32]	Level 2b	974(21), 504(17), **503(100),** 469(79), 161(17)	1.0	[M-H]—	
			12.73	958	Asiaticoside [30]	Level 2b	959(17), 958(26), 957(14), 488(33), **469(100)**	1.0	[M-H]—	
			20.05	520	Not identified	Level 5	521(59), 520(12), 503(10), 184(94), **104(100)**	1.0	[M+H]+	
7.	*Crocus sativus*	448,316,330,652,328,814,669,519	5.45	449	Astragalin [26]	Level 2b	451(8), 450(23), **287(100**), 449(90), 288(17)	1.0	[M+H]+	100
			6.87	317	Not identified	Level 2b	318(2), 156(11), **155(100),** 137(7), 85(1)	1.0	[M+H]+	
			8.05	348	Picrocrocin [26]	Level 2b	348(9), 170(10), **169(100),** 151(7), 123(2)	1.0	$[M+NH_4]+$	
			10.77	670	beta-D-gentiobiosyl crocetin [26]	Level 2b	670(7), 330(21), **329(100),** 311(34), 293(8)	1.0	$[M+NH_4]+$	
			14.07	346	Crocetin gentiobiosylglucosyl ester [26]	Level 2b	832(42), 487(24), **329(100),** 325(68), 311(39)	1.0	$[M+NH_4]+$	
			13.51	670	Not identified	Level 5	670(8), 330(19), **329(100),** 312(8), 311(37)	1.0	[M+H]+	
			18.74	520	Not identified	Level 5	520(2), 185(5), **184(100),** 104(3), 86(1)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	#Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
8.	*Curcuma longa*	216,392,390,438,542,368,495	10.64	217	Turmerone [26]	Level 2b	217(64), 161(19), 135(21), 121(33), **119(100)**	1.0	[M+H]+	100
			11.36	393	Not identified	Level 5	375(26), **357(100),** 179(55), 153(22), 137(28)	1.0	[M+H]+	
			12.29	391	Not identified	Level 5	392(20), 355(18), 197(17), **179(100),** 177(14)	1.0	[M+H]+	
			14.02	437	Not identified	Level 5	438(5), 437(21), 306(9), **305(100),** 149(6)	1.0	[M-H]—	
			15.26	541	Didemethoxybisabolocurcumin ether [33]	Level 2b	543(5), 541(38), 392(11), **391(100),** 390(16)	1.0	[M-H]—	
			15.98	369	Curcumin [34]	Level 2b	369(31), 285(22), 245(21), **177(100),** 175(21)	1.0	[M+H]+	
			21.66	496	Not identified	Level 5	497(7), 496(43), 478(9), 184(83), **104(100)**	1.0	[M+H]+	
9.	*Ficus religiosa*	396,426,236,201,370,495	3.00	397	Not identified	Level 5	361(7), **235(100),** 217(11), 133(6), 59(6)	1.0	[M+H]+	100
			4.92	236	Not identified	Level 5	237(6), 220(4), 219(32), 178(11), **177(100)**	1.0	[M+H]+	
			5.26	426	Not identified	Level 5	428(5), 427(24), 266(10), **265(100),** 247(11)	1.0	[M+H]+	
			10.80	201	Not identified	Level 5	202(35), 143(9), **142(100),** 100(7), 86(7)	1.0	[M+H]+	
			14.78	370	Not identified	Level 5	299(41), 203(91), **169(100),** 127(57), 97(28)	1.0	[M+H]+	
			19.97	496	Not identified	Level 5	497(9), 496(36), **104(100),** 478(7), 184(69)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
10.	*Morus alba*	178,626,386,640,464,478,356,648	4.00	179	5,7-Dihydroxychromone [26]	Level 2b	179(11), 133(37), **123(100),** 105(21), 77(24)	1.0	[M+H]+	100
			5.00	627	Kuwanon L [26]	Level 2b	466(18), **465(100),** 464(59), 303(46), 302(6)	1.0	[M+H]+	
			6.09	404	Not identified	Level 5	404(11), 243(11), **225(100),** 207(23), 189(8)	1.0	$[M+NH_4]+$	
			7.92	641	Not identified	Level 5	623(24), **479(100),** 478(42), 461(20), 181(30)	1.0	[M+H]+	
			8.92	465	Isoquercitrin [26]	Level 2b	465(10), 464(1), 304(6), **303(100),** 302(7)	1.0	[M+H]+	
			9.39	477	Not identified	Level 5	478(16), 477(33), 316(8), **315(100),** 179(27)	1.0	[M-H]—	
			15.77	357	Not identified	Level 5	339(19), 221(13), 207(61), **203(100),** 163(20)	1.0	[M+H]+	
			21.50	649	Chalcomoraci [26]	Level 2b	650(21), 649(33), 407(29), 271(28), **205(100)**	1.0	[M+H]+	
11.	*Myristica fragrans*	344,326,208,374,356,370,324	15.86	343	Nectandrin-B [26]	Level 2b	328(13), 310(13), 164(16), **151(100),** 136(17)	1.0	[M-H]—	100
			15.93	327	Licarin A [26]	Level 2b	327(21), 203(45), 165(15), **151(100),** 137(65)	1.0	[M+H]+	
			16.25	209	Elemecin [26]	Level 2b	194(32), 179(6), 169(9), **168(100),** 153(28)	1.0	[M+H]+	
			16.61	373	Fragransol A [26]	Level 2b	373(6), 209(2), 193(5), 180(12), **179(100)**	1.0	[M-H]—	
			18.80	357	Fragransol C [26]	Level 2b	**203(100),** 193(42), 188(20), 181(27), 171(22)	1.0	[M+H]+	
			21.22	371	Fragransol D [26]	Level 2b	**203(100),** 195(19), 193(28), 188(15), 181(15)	1.0	[M+H]+	
			22.43	325	Licarin B [26]	Level 2b	**203(100),** 188(56), 171(50), 161(44), 135(56)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
12.	*Nigella sativa*	204,286,772,304,164, 454,750,676,222	2.75	205	beta-Bisabolene [26]	Level 2b	206(3), 205(22), 189(12), **188 (100),** 146(2)	1.0	[M+H]+	100
			6.43	287	Kaempferol [34]	Level 2b	287(53), 165(30), **153(100),** 121(60), 69(58)	1.0	[M+H]+	
			6.50	773	Kaempferol 3,7,4′-triglucoside [35]	Level 2b	773(16), 449(16), 325(29), 288(12), **287(100)**	1.0	[M+H]+	
			8.30	305	Arachidonic Acid [26]	Level 2b	305(18), 248(16), **247(100),** 59(35), 58(5)	1.0	[M+H]+	
			9.48	165	Thymoquinone, Eugenol [26]	Level 2b	138(6), **137(100),** 124(8), 109(7), 81(10)	1.0	[M+H]+	
			12.18	455	Not identified	Level 5	409(62), 203(68), **189(100),** 177(41), 111(70)	1.0	[M+H]+	
			12.19	751	alpha-Hederin [26]	Level 2b	752(35), 657(50), 455(96), 438(74), **279(100)**	1.0	[M+H]+	
			23.24	677	Not identified	Level 5	554(35), 283(45), 265(22), 131(49), **124(100)**	1.0	[M+H]+	
			25.63	223	Dillapiol [26]	Level 2b	95(58), 83(83), 81(55), **69(100),** 55(45)	1.0	[M+H]+	
13.	*Ocimum tenuiflorum*	226,360,462,312,313,444,506	2.09	227	Not identified	Level 5	209(25), 191(33), 167(26), 149(34), **85(100)**	1.0	[M+H]+	100
			5.35	359	Not identified	Level 5	359(11), 197(54), 179(8), 162(8), **161(100)**	1.0	[M-H]—	
			5.99	463	Luteolin 7-glucuronide [3]	Level 2b	464(10), 463(17), 288(11), **287(100),** 121(1)	1.0	[M+H]+	
			9.42	311	Dihydroxy-octadecadienoic acid [36]	Level 2b	312(2), 311(11), 191(0), 150(6), **149(100)**	1.0	[M-H]—	
			10.75	314	Not identified	Level 5	315(3), 314(17), 178(7), **177(100),** 121(9)	1.0	[M+H]+	
			16.21	445	Not identified	Level 5	446(3), 445(10), 252(4), 195(13), **194(100)**	1.0	[M+H]+	
			17.96	507	Isoquercetrin acetate [37]	Level 2b	507(39), 257(18), **256(100),** 239(16), 238(75)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
14.	*Phyllanthus amarus*	221,276,418,354,260, 386,400,432,608	3.30	222	Not identified	Level 5	222(28), 148(6), 136(6), 79(6), **70(100)**	1.0	[M+H]+	100
			15.05	277	Not identified	Level 5	277(68), 135(97), 121(83), **93(100),** 79(65)	1.0	[M+H]+	
			17.42	419	Phyllanthin [25]	Level 2b	419(12), 388(20), 387(77), 356(21), **355(100**)	1.0	[M+H]+	
			17.49	355	Not identified	Level 5	355(42), 340(52), 177(30), 165(40), **151(100**)	1.0	[M+H]+	
			17.51	261	Not identified	Level 5	201(90), **173(100),** 171(33), 158(37), 145(35)	1.0	[M+H]+	
			17.42	387	Not identified	Level 5	388(5), 387(21), 356(23), **355(100),** 151(10)	1.0	[M+H]+	
			18.30	401	Lintetralin [25]	Level 2b	402(7), 401(29), 370(23), **369(100),** 165(3)	1.0	[M+H]+	
			18.30	433	Niranthin [37]	Level 2b	433(16), 402(23), 401(92), 370(22), **369(100)**	1.0	[M+H]+	
			20.49	609	Not identified	Level 5	559(42), 532(36), **531(100),** 515(36), 485(36)	1.0	[M+H]+	
15.	*Piper betel*	192,176,150,174,234,165,206	14.25	191	Not identified	Level 5	162(27), **161(100),** 149(36), 134(31), 122(27)	1.0	[M-H]—	100
			15.49	177	4-Allylphenyl acetate [25]	Level 2b	177(25), **149(100),** 122(6), 121(5), 119(12)	1.0	[M+H]+	
			15.74	151	Allylpyrocatechol [25]	Level 2b	133(8), **123(100),** 110(29), 105(31), 79(7)	1.0	[M+H]+	
			15.71	175	Not identified	Level 5	175(45), **128(100),** 127(29), 91(34), 77(32)	1.0	[M+H]+	
			15.72	252	4-Allyl-1,2-diacetoxybenzene [25]	Level 2b	194(11), 193(87), 175(25), 157(15), **151(100)**	1.0	$[M+NH_4]+$	
			16.15	166	Not identified	Level 5	138(21), 134(16), **125(100),** 124(35), 106(30)	1.0	[M+H]+	
			16.16	207	Eugenyl acetate [38]	Level 2b	189(82), 174(49), **165(100),** 157(52), 137(76)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	#Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
16.	*Piper longum*	265,316,150,222,367,208,220,273	1.28	266	Not identified	Level 5	248(43), 116(46), **98(100),** 87(84), 86(88)	1.0	[M+H]+	100
			3.04	317	Not identified	Level 5	281(28), 263(51), 191(30), **179(100),** 137(88)	1.0	[M+H]+	
			5.62	151	4-methoxyacetophenone [26]	Level 2b	151(32), 135(26), 108(29), **107(100),** 79(25)	1.0	[M+H]+	
			6.34	223	Delta-cardinol [26]	Level 2b	224(1), 223(1), 195(2), 182(10), **181(100)**	1.0	[M+H]+	
			11.56	368	Piperundecalidine [26]	Level 2b	368(7), 223(17), 128(21), 182(9), **181(100)**	1.0	[M+H]+	
			12.98	209	isopropyl-1,6-dimethyldecahydronaphthalene [26]	Level 2b	209(54), 194(53), 176(93), 167(61), **148(100)**	1.0	[M+H]+	
			13.48	221	Not identified	Level 5	221(53), 193(57), 191(44), **190(100),** 178(29)	1.0	[M+H]+	
			15.80	274	Piperlonguminine [26]	Level 2b	**201(100),** 171(13), 143(12), 135(95), 57(17)	1.0	[M+H]+	
17.	*Piper nigrum*	261,257,327,329,341,235,343	13.09	262	Not identified	Level 5	262(13), 178(10), **177(100)** 145(18), 86(19)	1.0	[M+H]+	100
			14.22	258	Coumaperine [26]	Level 2b	258(10), 174(11), **173(100),** 107(8), 86(8)	1.0	[M+H]+	
			17.86	328	Not identified	Level 5	328(34), 167(26), **161(100),** 131(56), 107(17.18)	1.0	[M+H]+	
			18.57	330	Not identified	Level 5	330(16), 135(47), 98(11), **72(100),** 70(21)	1.0	[M+H]+	
			19.59	342	Pipericide [26]	Level 2b	342(20), 229(21), 135(32), 112(17), **86(100)**	1.0	[M+H]+	
			19.67	236	Not identified	Level 5	236(31), 95(41), 86(50), 81(92), **69(100)**	1.0	[M+H]+	
			20.49	344	Piperolein B [26]	Level 2b	344(16), 135(40), 98(9), **86(100),** 84(20)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
18.	*Pongamia pinnata*	314,396,352,410,336, 326,380,366,350	11.57	315	Ponganone-vii [26]	Level 2b	316(4), 315(22), 298(18), **297(100),** 145(1)	1.0	[M+H]+	100
			14.66	397	Pongapinone B, Glabrachalcone [26]	Level 2b	379(57), 363(37), 361(39), 327(39), **309(100)**	1.0	[M+H]+	
			15.12	353	Pongagallone A [26]	Level 2b	354(8), 353(27), 339(19), **338(100),** 337(7)	1.0	[M+H]+	
			15.84	411	Pongapinone [26]	Level 2b	411(45), 351(53), 323(22), 298(17), **297(100)**	1.0	[M+H]+	
			15.94	337	Pongapin, Gamatin [26]	Level 2b	338(4), 337(21), 323(21), **322(100**), 176(5)	1.0	[M+H]+	
			16.25	327	Demethoxykanugin [26]	Level 2b	328(6), 327(37), 313(17), **312(100),** 311(5)	1.0	[M+H]+	
			16.79	381	Glabra chromene 1 [26]	Level 2b	363(9), 327(20), **309(100),** 219(5), 187(4)	1.0	[M+H]+	
			16.92	367	3- Methoxypongapin [26]	Level 2b	368(8), 367(35), 353(21), **352(100),** 351(10)	1.0	[M+H]+	
			19.66	351	Ovalichromene B [26]	Level 2b	351(10), 217(5), 204(13), **203(100),** 175(8)	1.0	[M+H]+	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	#Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
19.	*Saraca asoca*	578,290,448,432,463,268,304,519	6.82	579	Procyanidin B2 [26]	Level 2b	579(71), 427(64), **291(100),** 289(50), 127(35)	1.0	[M+H]+	100
			7.38	291	Catechin [26]	Level 2b	291(20), 165(35), 147(8), **139(100),** 123(58)	1.0	[M+H]+	
			8.57	303	Quercetin [26]	Level 2b	**285(100),** 217(15), 180(17), 177(40), 125(41)	1.0	[M-H]—	
			9.65	449	Quercetin-3-rhamnoside [26]	Level 2b	304(15), **303(100),** 147(11), 129(8), 85(8)	1.0	[M+H]+	
			10.65	433	Cosmosin [26]	Level 2b	288(14), **287(100),** 147(12), 129(10), 85(11)	1.0	[M+H]+	
			13.96	464	Quercetin 3-glucoside [26]	Level 2b	464(24), 210(10), **209(100),** 194(9), 193(86)	1.0	[M+H]+	
			16.70	269	Not identified	Level 5	209(23), 181(96), **163(100),** 135(27), 107(38)	1.0	[M+H]+	
			32.36	518	Not identified	Level 5	517(5), 384(13), 91(12), 60(2), **59(100)**	1.0	[M-H]—	

(Continues)

TABLE 2 | (Continued)

S. No.	Plant name	Chemical barcodes	Rt (±0.8) in Min.	Precursor ion ES (±)	Identified metabolites (References)	[#]Identification level [23]	MS/MS Fragment ions (Intensity in %)	[a]MS2 Score (%)	Annotation (Hits)	Accuracy (%)
20.	*Vitex negundo*	496,466,360,346,374, 388,377,270,272	3.53	495	Negundoside [26]	Level 2b	213(17), 169(33), 151(37), **137(100),** 125(22)	1.0	[M-H]—	100
			7.31	465	Agnuside [26]	Level 2b	285(81), **137(100),** 136(32), 101(20), 89(34)	1.0	[M-H]—	
			14.23	361	chrysoplenol D [26]	Level 2b	285(42), 303(49), **328(100),** 345(82), 311(43)	1.0	[M+H]+	
			15.13	347	Aucubin, trimethoxyflavone [26]	Level 2b	347(8), 198(9), **197(100),** 182(3), 177(20)	1.0	[M+H]+	
			15.42	375	Casticin [26]	Level 2b	359(86), 345(40), 342(53), **317(100),** 299(77),	1.0	[M+H]+	
			16.54	389	Artemetin [26]	Level 2b	373(50), 359(47), 356(43), **331(100),** 313(53)	1.0	[M+H]+	
			19.29	378	Not identified	Level 5	**300(100),** 283(72), 257(42), 241(41), 72(49)	1.0	[M+H]+	
			21.13	271	Not identified	Level 5	159(75), 145(72), 95(73), **81(100),** 69(71)	1.0	[M+H]+	
			21.40	273	Glucononitol [26]	Level 2b	163(82), 109(74), **95(100),** 81(80), 69(85)	1.0	[M+H]+	

Note: Values in bold emphasis are the major characteristic fragment.
[a]MS2 score: 1 = 100% matched (Top 5 fragments, based on intensity); hits: annotation of precursor ion.
Identification level as per Scymanski et al. (2014).

3.3.5 | *Vitex negundo*

The Table 2 highlights the chemical characterization of various compounds identified in *V. negundo*. Negundoside (495 *m/z*) was detected at 3.53 min with a notable MS2 match, followed by agnuside (465 *m/z)* at 7.31 min. Chrysosplenol D (361 *m/z*) appeared at 14.23 min, while aucubin and trimethoxyflavone (347 *m/z*) were observed at 15.13 min. Casticin (375 *m/z*) and artemetin (389 *m/z*) were identified at 15.42 and 16.54 min, respectively. At later retention times, compounds with *m/z* values of 378 (19.29 min), 271 (21.13 min), and 273 (21.40 min) were detected but remained unidentified except for gluconitol (273 *m/z*) (Table 2). The MS2 scores for all compounds suggest high confidence in fragment matches, signifying precise annotation of precursor ions and ensuring reliable identification of these metabolites.

4 | Conclusion

The chemical barcode of selected botanicals and the web-based LC–MS/MS library of nontargeted plant secondary metabolites were successfully utilized for the identification of plant species. We followed a SOP from sample preparation to library search. This approach facilitated the identification of plant species through their distinctive molecular weight sequences (chemical barcode) and MS/MS fingerprint of metabolites. Thus, the plant metabolome library could serve as a reference library for botanical identification with its chemical integrity.

Author Contributions

Akhilesh Kumar: software, formal analysis, data curation, visualization, validation, methodology, writing – original draft, writing – review and editing, investigation. **Mohsin Ali:** resources, formal analysis. **Avinash Kumar:** resources, formal analysis. **Dipak Kumar Mishra:** resources, supervision, validation, methodology. **Sanjeev Kanojiya:** conceptualization, investigation, funding acquisition, writing – review and editing, supervision, resources, methodology, software, project administration.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Peer Review

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Supporting Information

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