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Research article

Rapid chemical characterization and pharmacological mechanism of Fining Granules in the treatment of chronic bronchitis based on UHPLC–Q-exactive orbitrap mass spectrometer and network pharmacology

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ABSTRACT

Background: Senecio cannabifolius Less. is a perennial herb belonging to the Compositae family that has been used in traditional medicine as an antitussive and expectorant for treating chronic bronchitis and acute respiratory infections. Traditionally, Feining Granules are prepared from water extracts of the raw plant material. However, the chemical composition and pharmacological mechanisms of Feining Granules have not been thoroughly investigated.

Methods: A systematic strategy for the rapid detection and identification of the constituents of Feining Granules was developed using ultrahigh-performance liquid chromatography–quadrupole-exactive orbitrap mass spectrometry (MS) with parallel reaction monitoring.

Results: Overall, 162 compounds, including flavonoids, alkaloids, organic acids, and others, were identified unambiguously and tentatively by comparing the retention times and MS fragmentation with reference standards and literature data. Ninety-nine of these were reported for the first time to the best of our knowledge. Network pharmacology suggests that Feining Granules can be used to treat chronic bronchitis as they contain active components associated with the *ALB*, *VEGFA*, and *SRC* target genes influenced by HIF-1, VEGF, and other signaling pathways.

Conclusion: These results provide information that can help understand the effective substances of *S. cannabifolius* Less. and improve quality control.

1. Introduction

Senecio cannabifolius Less. a perennial herb belonging to the Compositae family, is distributed widely in Northeast China, Japan, and Korea. The rhizomes of this plant have been used in traditional medicine for their antitussive and expectorant properties, particularly in treating chronic bronchitis and acute respiratory infections [1–5]. Conventionally, Feining Granules are prepared from the water extracts of the raw plant material. Although a previous investigation highlighted the presence of organic acids, alkaloids, and

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flavonoids in this plant, a comprehensive exploration of the chemical composition and pharmacological mechanisms of Feining Granules is lacking, warranting further investigation. In recent decades, suitable analytical methods for identifying medicinal plants require high separation efficiency, selectivity, and sensitivity. The substances are highly complex, and the relevant compounds might be present only in trace amounts or accompanied by numerous other compounds with similar structures. Therefore, techniques such as thin-layer chromatography or UV–vis spectrophotometry are not the optimal choice in most cases [6]. Liquid chromatography–mass spectrometry (LC–MS) has become the gold standard for rapidly identifying chemical compounds in drug or biological samples. In particular, ultrahigh-performance LC (UHPLC)–MS has played a pivotal role in detecting constituents of traditional Chinese medicine owing to its heightened sensitivity and selectivity [7,8]. Various studies have used LC–MS to improve detection capabilities for traditional medicinal plant [9,10]. Network pharmacology is recognized for its cost-effectiveness in drug development and is a promising approach for studying compound–proteins/genes–disease pathways, such as the pharmacological mechanisms of traditional Chinese medicine [11]. This technique offers valuable insights into analysis of complex plant metabolites and Quality control.

Herein, the chemical and pharmacological mechanisms of Feining Granules in the treatment of chronic bronchitis were systematically characterized using UHPLC–quadrupole (UHPLC–Q)-exactive orbitrap MS and network pharmacology. Overall, 162 compounds were identified unambiguously and tentatively, including flavonoids, alkaloids, organic acids, and other compounds. These compounds were found to influence target genes, such as *ALB*, *VEGFA*, and *SRC*, acting through pathways involving HIF-1, VEGF, and others to exert their efficacy. These findings help better understand the pharmacological properties and mechanisms of Feining Granules, paving the way for future research, including quality control measures.

2. Materials and methods

2.1. Reagents, chemicals, and materials

In addition to the MS-grade formic acid purchased from ThermoFisher Scientific China, chromatographic-grade methanol and acetonitrile were provided by Merck (New Jersey, USA). Watson's water (Guangzhou, China) was used as the mobile phase. The other solvents were analytical grade. The reference standards of salicylic acid, D-(−)-quinic acid, and gallic acid were provided by Shanghai Yuanye Bio-Technology Co., Ltd. Rutin, hyperoside, and quercetin were obtained from Chengdu Efa Biotechnology Co., Ltd. Isoquercitrin and luteolin 7-O-D-glucoside were purchased from Chengdu Ruifen Biotechnology Co., Ltd. Citric acid, L-(−)-malic acid, caffeic acid, 4-hydroxycinnamic acid, octanedioic acid, and azelaic acid were provided by Shandong XiAsia Chemical Co., Ltd. 3,4-Dicaffeoylquinic acid lactones, 3,5-dicaffeoylquinic acid lactones, and 4,5-dicaffeoylquinic acid lactones were supplied by Chengdu Herbpurify Co., Ltd.

2.2. Standard and sample preparation

Each reference standard was weighed precisely and dissolved in methanol. Extraction with 70 mL of 70 % aqueous ethanol was performed for 30 min on Feining Granule powder (1 g) using sonication. The extracted solution was filtered and dried under reduced pressure to yield brown residues with 23.1 ± 1.1 % (n = 3) extraction rate. One milliliter of methanol was -dissolved and centrifuged (15 min, 4 °C, 10000 rpm) to obtain the supernatant for further analysis.

2.3. Instrument and conditions

The Thermo Q-exactive focus orbitrap MS combined with Thermo Scientific Dionex Ultimate 3000 RS (Thermo Fisher Scientific, California, USA) was used for LC–MS analysis. Sample separation was performed on Hypersil GOLD aQ (100 mm × 2.1 mm, 1.9 μm) at 40 °C at a flow rate of 0.3 mL/min. The mobile phase comprised water containing 0.1 % formic acid (A) and acetonitrile containing 0.1 % formic acid (B) in the following gradient: 0 min, 5 % B; 2 min, 8 % B; 5 min, 12 % B; 10 min, 22 % B; 12 min, 40 % B; 20 min, 70 % B; 25 min, 95 % B; 26 min, 5%B; and 30 min, 5%B. The injection volume was 2 μL.

All samples underwent electrospray ionization (ESI) with positive and negative ionization modes over the scan range of 120–1000 *m/z*. The sheath and auxiliary gases were 30 and 10, respectively. The spray voltage was 3.5 kV for (+)-ESI and 3.0 kV for (−)-ESI. The capillary and auxiliary gas heater temperatures were 320 °C and 350 °C, respectively. The MS^1 spectra were acquired in full MS mode at a resolution of 35,000, and the MS^2 spectra were obtained via ddMS^2 triggered by the intensity of Top3. The normalized collision energy (NEC) was 30 %, with 5.0 × e^5 of the automatic gain control target.

2.4. Data processing process

All high-resolution MS data were acquired and processed using Xcalibur™ version 4.1 on Compound Discovery 3.0 version (Thermo Fisher Scientific, California, USA). The data were imported into a CD with the traditional Chinese medicine workflow template to identify the chemicals by comparing the MS information with the MS database, including the mzCloud and Orbitrap Traditional Chinese Medicine Library (OTCML). There was a maximum mass tolerance of 10 ppm for MS^1 and MS^2, a minimum peak intensity of 500,000, and a maximum element of $C_{60}H_{120}O_{60}N_{10}$.

2.5. Screening of effective components of Feining Granules

The effective components were screened using the criteria including oral drug bioavailability (OB) of ≥0.18 and a kind of medicinal property (DL) of ≥30 % of those compounds identified via UHPLC–Q-exactive focus MS. The OB and DL values were obtained from the

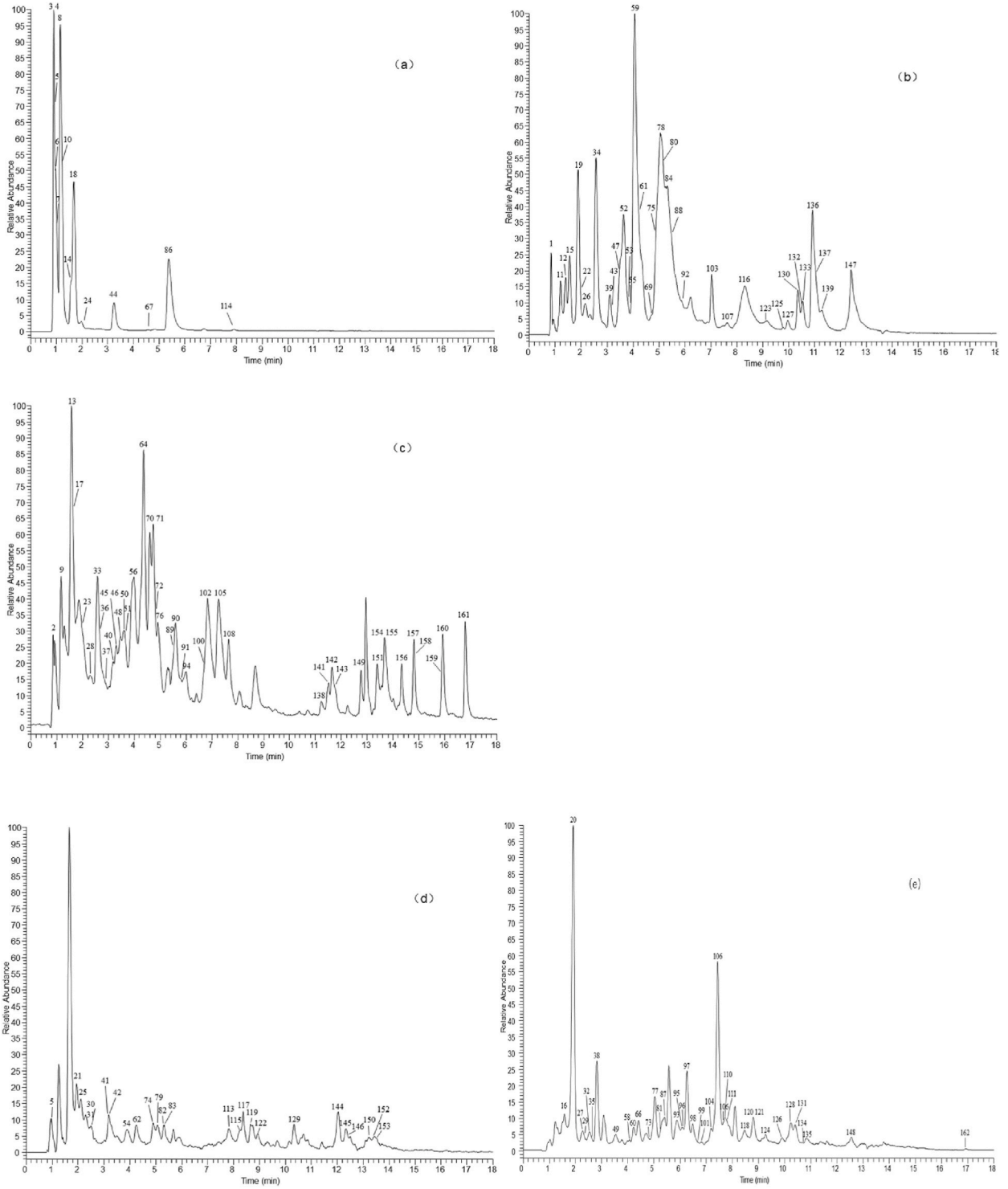

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Fig. 1. High-resolution extracted ion chromatograms (EIC) of the Lemna minor extract. (a–d) for negative and (e) for positive ion motion mode): (a) 353.0878, 195.0510, 173.0819, 171.0662, 191.0561, 191.0197 (b) 213.0768, 315.0721, 177.0193, 303.0874, 515.1195, 463.0881, 179.0349, 137.0244, 147.0298, 415.1034, 181.0717, 175.0611, 187.0975, 167.0349, 231.0863, 231.0874, 153.0193 (c) 159.0662, 299.0772, 431.0983, 267.0662, 263.1288, 165.0557, 229.1445, 339.0721, 447.0932, 341.0878, 497.1089, 173.0455, 163.0400, 269.0455, 293.1758, 341.1089, 593.1300, 329.0878, 325.0928, 253.0506, 169.0142, 243.0622, 337.0928, 181.0506, 151.0400 (d) 593.1511, 301.0353, 505.0987, 499.1245, 259.0248, 153.0557, 477.1038, 367.1034, 515.1406, 609.1461, 265.1445, 537.1977, 197.0455 (e) 256.1543, 354.1911, 373.1281, 139.0389, 449.1078, 368.1703, 388.1521, 183.0651, 197.0808, 336.1805, 334.1648, 238.1437, 240.1594, 370.1860, 338.1961, 251.1277, 254.1386, 350.1598, 223.1328, 352.1754, 225.1485, and 366.1547.

Traditional Chinese Medicine Database and analysis platform System Pharmacology (TCMSP, http://tcmspw.com/tcmsp.php). The potential targets of the effective components were achieved by SwissTargetPrediction (http://www.swisstargetprediction.ch/) and TCMSP.

2.6. Screening of drug–disease common targets

The target of chronic bronchitis was obtained from GeneCards (https://www.genecards.org/) and disgenet (https://www.disgenet.org) database with the keyword "chronic bronchitis." The drug–disease common targets were obtained using Venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/).

2.7. Protein–protein interaction network

The protein–protein interaction (PPI) network was performed on the STRING11.5 (https://cn.string-db.org/) database by uploading the drug–disease common target. The species was selected as "HOMO sapiens" with a confidence score of >0.4. Cytoscape (https://cytoscape.org) was used to construct the PPI network and screen core targets.

2.8. Kyoto Encyclopedia of Genes and Genomes pathway analysis and GO enrichment analysis of the cotarget

GO enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were achieved using the DAVID database (https://david.ncifcrf.gov/) with the species "*Homo sapiens*."

2.9. Construction of the drug–component–target–pathway network

A "drug–component–target–pathway" interaction network visualization was analyzed using Cytoscape software.

3. Result and discussion

3.1. Chemical identification in Feining Granules

The entire extract and its components were analyzed via UHPLC–Q-exactive orbitrap MS (Fig. 1(a–e)). Overall, 162 compounds were identified (Table 1), including 95 organic acids, 20 flavonoids, 26 alkaloids, and 21 other types of compounds.

3.1.1. Identification by comparing with standards

Based on the retention time, high-resolution MS, and other techniques, 21 chemicals were identified accurately from the fragment ions and reference substances, including quinic acid (3), L-(−)-malic acid (5), citric acid (8), gallic acid (13), 3-caffeoylquinic acid (44), 5-caffeoylquinic acid (85), caffeic acid (88), 1,3-dicaffeoylquinic acid lactones (107), 4-hydroxycinnamic acid (108), octanedioic acid (114), salicylic acid (123), rutin (129), hyperoside (130), isoquercitrin (133), luteoloside (134), azelaic acid (136), 3,4-dicaffeoylquinic acid lactones (137), 3,5-dicaffeoylquinic acid lactones (139), 4,5-dicaffeoylquinic acid lactones (147), abscisic acid (149), and quercetin (153).

3.1.2. Organic acids

Compound 6 has the same primary and secondary MS information as compound 8 (citric acid). Therefore, it was identified as isocitric acid. Similarly, compounds 89 and 91, 92 and 116, and 143 were proposed to be hydroxyl benzeneacetic acid isomers [12], caffic acid isomers [13], and an abscisic acid isomer, respectively.

Five compounds, 7, 10, 14, 24, and 67, displayed the quasi-molecular ion at *m/z* 173.0819 and fragment ions at *m/z* 111.0072 and *m/z* 59.0123, which were tentatively labeled as 2-(1,4-dihydroxycyclohexanyl)-acetic acidisomers [12]. Similarly, compounds 59, 78, 84, and 86 were identified as 5-ethylidene-2-hydroxy- 2-hydroxymethyl-3-methylhexane-dioic acid or its isomers [12]. Compounds 22 and 65 and compounds 36 and 50 were identified as seneciphyllic acid isomers and benzeneacetic acid isomers, respectively [12].

Compounds 12, 41, 49, 69, 109, 160, 15, 17, 19, 29, 34, 39, and 55 were identified as dihydroxyl phenylacetic acid, hydroxytyrosol, hydroxyl benzoic acid, protosappanin B, syringaldehyde, 6-gingerol, homogentisic acid, shikimic acid, homoprotocatechuic acid, veratric acid, protocatechuic acid, vanillic acid isomers, hydroxyl benzoic acid, by combining with the databases.

Eight isomers, 28,37, 46, 48, 57, 63, 70, and 76, exhibited the deprotonated ion [M − H]−341.0878, which possessed the same

Table 1
Accurate mass data of the 162 compound standards determined using UHPLC–Q-exactive orbitrap mass spectrometer.

Peak	T_r	Theoretical mass *m/z*	Experimental mass *m/z*	Error (ppm)	Formula(M)	MS/MS fragment (+)	MS/MS fragment (−)	Proposed identity	Classification
1	0.86	181.0717	181.0705	−6.96	$C_6H_{14}O_6$		MS^2[181]: 101.0229(100), 89.0228 (96), 71.0123(65), 59.0123(60)	Mannitol	Others
2	0.86	341.1089	341.1080	−2.65	$C_{12}H_{22}O_{11}$		MS^2[341]: 89.0229(100), 59.0123 (44), 71.0123(35), 101.0229(34)	Lactose	Others
3	0.89[a]	191.0561	191.0548	−6.81	$C_7H_{12}O_6$		MS^2[191]: 85.0279(100), 87.0071 (39), 59.0123(21), 111.0072(17)	Quinic acid	Organic acid
4	0.89	195.0510	195.0497	−6.59	$C_6H_{12}O_7$		MS^2[195]: 75.0072(100), 129.0179 (83), 99.0072(27), 87.0072(21)	Glucopyranuronic acid	Organic acid
5	0.94[a]	133.0142	133.0127	−11.10	$C_4H_6O_5$		MS^2[133]: 115.0021(100), 71.0123 (44)	Malic acid	Organic acid
6	0.97	191.0197	191.0186	−5.78	$C_6H_8O_7$		MS^2[191]: 111.0072(100), 85.0279 (23)	Isocitric acid	Organic acid
7	1.05	173.0819	173.0806	−1.12	$C_8H_{14}O_4$		MS^2[173]: 111.0072(100), 59.0123 (37)	2-(1,4-Dihydroxycyclohexanyl)-acetic acid or its isomers	Organic acid
8	1.15[a]	191.0197	191.0184	−6.78	$C_6H_8O_7$		MS^2[191]: 111.0072(100), 87.0072 (34), 85.0279(23)	Citric acid	Organic acid
9	1.16	243.0622	243.0614	−3.16	$C_9H_{12}N_2O_6$		MS^2[243]: 110.0232(100)	Uridine	Others
10	1.21	173.0819	173.0806	−1.36	$C_8H_{14}O_4$		MS^2[173]: 111.0072(100), 59.0123 (45)	2-(1,4-Dihydroxycyclohexanyl)-acetic acid or its isomers	Organic acid
11	1.22	147.0298	147.0284	−9.77	$C_5H_8O_5$		MS^2[147]: 87.0072(100), 85.0279 (72), 129.0178(54)	Hydroxyglutaric acid	Organic acid
12	1.41	167.0349	167.0337	−7.43	$C_8H_8O_4$		MS^2[167]: 123.0437(100), 108.0202 (40)	Dihydroxyl phenylacetic acid	Organic acid
13	1.54[a]	169.0142	169.0129	−7.55	$C_7H_6O_5$		MS^2[169]: 69.0332(100), 125.0232 (47), 97.0282(47)	Gallic acid	Organic acid
14	1.56	173.0819	173.0806	−1.30	$C_8H_{14}O_4$		MS^2[173]: 59.0123(100), 113.0592 (14)	2-(1,4-Dihydroxycyclohexanyl)-acetic acid or its isomers	Organic acid
15	1.57	167.0349	167.0337	−7.61	$C_8H_8O_4$		MS^2[167]: 108.0201(100), 123.0437 (31)	Homogentisic acid	Organic acid
16	1.58	254.1386	254.1386	−0.17	$C_{13}H_{19}NO_4$	MS^2[254]: 254.1391(100), 138.0912(8)		7-Angeloylretronecine N-oxide	Alkaloids
17	1.64	173.0455	173.0441	−8.30	$C_7H_{10}O_5$		MS2[173]: 59.0123(100), 113.0592 (15)	Shikimic acid	Organic acid
18	1.68	171.0662	171.0649	−1.43	$C_8H_{12}O_4$		MS^2[171]: 59.0123(100), 109.0644 (19), 111.0436(16)	Tetrahydro-jacaranone	Others
19	1.89	167.0349	167.0336	−7.73	$C_8H_8O_4$		MS^2[167]: 123.0437(100)	Homoprotocatechuic acid	Organic acid
20	1.91	370.1860	370.1859	−0.32	$C_{18}H_{27}NO_7$	MS^2[370]: 370.1859(100), 120.0807(2), 138.0913(2)		Jacoline	Alkaloids
21	1.95	259.0248	259.0275	10.49	$C_{13}H_8O_6$		MS^2[259]: 215.0374(100)	Tetrahydroxyxanthone	Others
22	1.97	213.0768	213.0759	−4.11	$C_{10}H_{14}O_5$		MS^2[213]: 169.0491(100), 169.0979 (44)	Seneciphyllic acid isomers	Organic acid
23	1.97	299.0772	299.0765	−2.24	$C_{13}H_{16}O_8$		MS^2[299]: 137.0230(100), 93.0330 (34)	Salicylic acid-hexoside	Others
24	1.98	173.0819	173.0807	−0.667	$C_8H_{14}O_4$		MS^2[173]: 59.0124(100), 113.0593 (13)	2-(1,4-Dihydroxycyclohexanyl)-acetic acid or its isomers	Organic acid
25	2.12	197.0455	197.0446	−4.80	$C_9H_{10}O_5$		MS^2[197]: 135.0437(100)	Salvianic acid A	Organic acid
26	2.18	315.0721	315.0717	−1.28	$C_{13}H_{16}O_9$		MS^2[315]: 153.0180(100), 109.0281 (50)	Protocatechuic acid-O-hexoside	Others

(*continued on next page*)

Table 1 (*continued*)

Peak	T_r	Theoretical mass *m/z*	Experimental mass *m/z*	Error (ppm)	Formula(M)	MS/MS fragment (+)	MS/MS fragment (−)	Proposed identity	Classification
27	2.28	254.1386	254.1386	0.02	$C_{13}H_{19}NO_4$	MS^2[254]: 140.1069(100)		9-Angeloylretronecine N-oxide	Alkaloids
28	2.29	341.0878	341.0871	−1.98	$C_{15}H_{18}O_9$		MS^2[341]: 161.0231(100), 179.0339 (49), 61.9870(37), 135.0437(34)	Caffeoyl hexoside	Organic acid
29	2.36	183.0651	183.0652	0.51	$C_9H_{10}O_4$	MS^2[183]: 113.9639(100), 131.9743(46), 159.9690 (39), 141.9586(34), 72.9377(18)		Veratric acid	Organic acid
30	2.50	153.0557	153.0543	−1.57	$C_8H_{10}O_3$		MS^2[153]: 123.0436(100)	2-(3,4-Dihydroxyphenyl) ethyl alcohol	Others
31	2.50	515.1406	515.1395	−2.13	$C_{22}H_{28}O_{14}$		MS^2[515]: 179.0337(100), 191.0547 (24)	Caffeoylquinic acid-hexoside	Others
32	2.53	254.1386	254.1390	1.39	$C_{13}H_{19}NO_4$	MS^2[354]: 120.0810(18)		7-Angeloylretronecine N-oxide	Alkaloids
33	2.57	329.0878	329.0871	−1.96	$C_{14}H_{18}O_9$		MS^2[329]: 167.0336(100), 123.0437 (26)	Vanillic acid-hexoside	Others
34	2.58	153.0193	153.0179	−8.90	$C_7H_6O_4$		MS^2[153]: 109.0280(100)	Protocatechuic acid	Organic acid
35	2.61	368.1703	368.1707	1.00	$C_{18}H_{25}NO_7$	MS^2[368]: 138.0914(100), 120.0810(14)		Retrorsine N-oxide	Alkaloids
36	2.62	151.0400	151.0387	−1.59	$C_8H_8O_3$		MS^2[151]: 107.0487(100)	Hydroxyl benzeneacetic acid or its isomers	Organic acid
37	2.77	341.0878	341.0874	−1.18	$C_{15}H_{18}O_9$		MS^2[341]: 191.0549(100), 161.0234 (15)	Caffeoyl hexoside	Organic acid
38	2.81	366.1547	366.1547	0.16	$C_{18}H_{23}NO_7$	MS^2[366]: 366.1547(100), 120.0808(1), 138.0911(1)		Riddelliine N-oxide	Alkaloids
39	3.11	167.0349	167.0338	−6.83	$C_8H_8O_4$		MS^2[167]: 123.0438(100)	Vanillic acid isomers	Organic acid
40	3.16	181.0506	181.0495	−6.08	$C_9H_{10}O_4$		MS^2[181]: 163.0387(100), 135.0437 (62), 119.0489(34), 72.9916(36)	3-(4-Hydroxyphenyl) lactic acid	Organic acid
C	3.19	153.0557	153.0544	−8.54	$C_8H_{10}O_3$		MS^2[153]: 109.0280(100)	Hydroxytyrosol	Organic acid
42	3.20	515.1406	515.1397	−1.66	$C_{22}H_{28}O_{14}$		MS^2[515]: 179.0338(100), 191.0551 (35)	Caffeoylquinic acid-hexoside	Organic acid
43	3.23	179.0349	179.0332	−9.45	$C_9H_8O_4$		MS^2[179]: 135.0438(100)	Dihydroxy cinnamic acid	Organic acid
44	3.24[a]	353.0878	353.0871	−1.82	$C_{16}H_{18}O_9$		MS^2[353]: 191.0550(100), 179.0338 (77), 135.0438(23)	3-Caffeoylquinic acid	Organic acid
45	3.30	197.0455	197.0445	0.30	$C_9H_{10}O_5$		MS^2[197]: 153.0543(100), 182.0210 (39)	Syringic acid	Organic acid
46	3.30	341.0878	341.0869	−2.42	$C_{15}H_{18}O_9$		MS^2[341]:135.0438(100)	Caffeoyl hexoside	Organic acid
47	3.44	231.0863	231.0864	0.54	$C_{10}H_{14}O_6$		MS^2[231]: 185.0808(100), 167.0701 (52), 123.0801(42), 101.0229(30), 139.0751(17)	Cannabiside C	Others
48	3.47	341.0878	341.0873	−1.36	$C_{15}H_{18}O_9$		MS^2[341]: 135.0438(100), 179.0338 (50), 161.0231(22), 191.0550(13)	Caffeoyl hexoside	Organic acid
49	3.52	139.0389	139.0390	0.64	$C_7H_6O_3$	MS^2[139]: 111.0443(100), 93.0339(16)		Hydroxyl benzoic acid	Organic acid
50	3.60	151.0400	151.0388	−1.13	$C_8H_8O_3$		MS^2[151]: 107.0487(100)	Hydroxyl benzeneacetic acid or its isomers	Organic acid
51	3.64	163.0400	163.0389	−6.97	$C_9H_8O_3$		MS^2[163]: 119.0488(100)	Coumaric acid	Organic acid
52	3.65	175.0611	175.0600	−6.77	$C_7H_{12}O_5$		MS^2[175]: 115.0386(100), 85.0643 (37), 113.0593(37)	2-Isopropylmalic acid	Organic acid
53	3.83	153.0193	153.0181	−7.85	$C_7H_6O_4$		MS^2[153]: 109.0280(100), 108.0202 (26)	Gentisic acid	Organic acid

(*continued on next page*)

Table 1 (*continued*)

Peak	T_r	Theoretical mass *m/z*	Experimental mass *m/z*	Error (ppm)	Formula(M)	MS/MS fragment (+)	MS/MS fragment (−)	Proposed identity	Classification
54	3.89	515.1406	515.1405	−0.25	$C_{22}H_{28}O_{14}$		MS^2[515]: 191.0550(100), 96.9587 (23), 173.0447(13), 179.0333(11)	Caffeoylquinic acid-hexoside	Organic acid
55	3.92	137.0244	137.0231	−9.39	$C_7H_6O_3$		MS^2[137]: 93.0331(100)	Hydroxyl benzoic acid	Organic acid
56	3.95	339.0721	339.0715	−1.75	$C_{15}H_{16}O_9$		MS^2[339]: 177.0181(100)	Esculin hydrate	Others
57	4.02	341.0878	341.0872	−1.71	$C_{15}H_{18}O_9$		MS^2[341]: 179.0339(100), 135.0438 (32), 191.0551(16)	Caffeoyl hexoside	Organic acid
58	4.07	352.1754	352.1755	0.21	$C_{18}H_{25}NO_6$	MS^2[352]: 120.0805(100), 138.0909(65)		Integerrimine N-oxide	Alkaloids
59	4.08	231.0874	231.0866	−3.33	$C_{10}H_{16}O_6$		MS^2[231]: 187.0964(100), 187.0602 (70), 117.0179(64), 141.0908(58)	5-Ethylidene-2-hydroxy- 2-hydroxy-methyl-3-methylhexane-dioic acid	Organic acid
60	4.20	350.1598	350.1600	0.61	$C_{18}H_{23}NO_6$	MS^2[350]: 350.1595(100), 120.0810(6), 138.0913(2)		Riddelline	Alkaloids
61	4.20	179.0349	179.0338	−6.43	$C_9H_8O_4$		MS^2[179]: 135.0439(100)	Caffeic acid isomer	Organic acid
62	4.23	515.1406	515.1397	−1.66	$C_{22}H_{28}O_{14}$		MS^2[515]: 173.0443(100), 179.0339 (65), 191.053(29), 96.9587(20)	Caffeoylquinic acid-hexoside	Organic acid
63	4.25	341.0878	341.0875	−0.63	$C_{15}H_{18}O_9$		MS^2[341]: 179.0338(100), 221.0447 (36), 135.0438(28), 161.0229(24)	Caffeoyl hexoside	Organic acid
64	4.37	337.0928	337.0925	−1.07	$C_{16}H_{18}O_8$		MS^2[337]: 163.0388(100), 119.0488 (21)	4-Tran-3-O-*p*-coumaroylquinic acid, Tran-3-Tran-3-O-*p*-coumaroylquinic acid	Organic acid
65	4.41	213.0768	213.0758	−4.67	$C_{10}H_{14}O_5$		MS^2[213]: 169.0858(100)	Seneciphyllic acid isomers	Organic acid
66	4.42	388.1521	388.1522	0.27	$C_{18}H_{26}CLNO_6$	MS^2[388]: 388.1514(100), 120.0815(1), 138.0913(1)		Jaconine	Alkaloids
67	4.55	173.0819	173.0806	−0.84	$C_8H_{14}O_4$		MS^2[173]: 59.0124(100), 113.0594 (13)	2-(1,4-Dihydroxycyclohexanyl)-acetic acid or its isomers	Organic acid
68	4.57	159.0662	159.0650	−7.93	$C_7H_{12}O_4$		MS^2[159]: 115.0750(100), 159.0650 (50)	2-Ethyl-3-methylsuccinic acid	Organic acid
69	4.73	303.0874	303.0870	−1.32	$C_{16}H_{16}O_6$		MS^2[303]: 243.0657(100), 121.0281 (65), 135.0438(48), 109.0278(24)	Protosappanin B	Organic acid
70	4.73	341.0878	341.0871	−1.80	$C_{15}H_{18}O_9$		MS^2[341]: 179.0337(100), 135.0437 (30)	Caffeoyl hexoside	Organic acid
71	4.74	325.0928	325.0923	−1.694	$C_{15}H_{18}O_8$		MS^2[325]: 163.0388(100), 119.0488 (41)	Coumaroylhexose	Others
72	4.81	159.0662	159.0650	−7.87	$C_7H_{12}O_4$		MS^2[159]: 97.0644(100), 115.0750 (47),	Pimelic acid	Organic acid
73	4.85	256.1543	256.1541	−0.76	$C_{13}H_{21}NO_4$	MS^2[256]: 256.1542(100), 138.0912(24)		Creatonotine	Alkaloids
74	4.89	515.1406	515.1397	−1.66	$C_{22}H_{28}O_{14}$		MS^2[515]: 191.0550(100), 323.0765 (45), 179.0342(20), 161.0232(16), 96.9586(12)	Caffeoylquinic acid-hexoside	Organic acid
75	4.90	153.0193	153.0180	−8.24	$C_7H_6O_4$		MS^2[153]: 109.0280(100)	Dihydroxyl benzoic acid	Organic acid
76	4.91	341.0878	341.0875	−0.74	$C_{15}H_{18}O_9$		MS^2[341]: 179.0338(100), 135.0438 (21), 221.0443(14), 161.0230(8)	Caffeoyl hexoside	Organic acid
77	5.03	238.1437	238.1437	−0.08	$C_{13}H_{19}NO_3$	MS^2[238]: 138.0914(100)		7-Angeloylretronecine	Alkaloids
78	5.06	231.0874	231.0866	−3.42	$C_{10}H_{16}O_6$		MS^2[231]: 187.0600(100), 143.0700 (85)	5-Ethylidene-2-hydroxy-2-hydroxymethyl-3-methylhexane-dioic acid or its isomers	Organic acid
79	5.06	515.1406	515.1402	−0.83	$C_{22}H_{28}O_{14}$		MS^2[515]: 179.0337(100), 173.0443 (56), 191.0550(44), 341.0864(21)	Caffeoylquinic acid-hexoside	Organic acid

(*continued on next page*)

Table 1 (*continued*)

Peak	T_r	Theoretical mass *m/z*	Experimental mass *m/z*	Error (ppm)	Formula(M)	MS/MS fragment (+)	MS/MS fragment (−)	Proposed identity	Classification
80	5.17	177.0193	177.0186	0.38	$C_9H_6O_4$		MS^2[177]: 133.0284(100), 105.0333 (45), 149.0231(13)	Esculetin	Others
81	5.29	352.1754	352.1760	0.05	$C_{18}H_{25}NO_6$	MS^2[352]: 120.0806(100), 138.0909(32)		Jacobine	Alkaloids
82	5.29	515.1406	515.1402	−0.83	$C_{22}H_{28}O_{14}$		MS^2[515]: 191.0549(100), 179.0339 (39), 173.0443(24)	Caffeoylquinic acid-hexoside	Organic acid
83	5.32	367.1034	367.1030	−1.07	$C_{17}H_{20}O_9$		MS^2[367]: 193.0494(100), 149.0230 (21), 134.0359(17), 135.0438(13), 173.0447(13)	3-Feruloylquinic acid	Organic acid
84	5.33	231.0874	231.0864	−4.11	$C_{10}H_{16}O_6$		MS^2[231]: 187.0600(100), 143.0699 (100)	5-Ethylidene-2-hydroxy-2-hydroxymethyl-3-methylhexane-dioic acid or its isomers	Organic acid
85	5.36[a]	353.0878	353.0869	−2.45	$C_{16}H_{18}O_9$		MS^2[353]: 173.0443(100), 179.0338 (69), 191.0550(65), 135.0437(32)	5-caffeoylquinic acid	Organic acid
86	5.37	173.0819	173.0806	−1.30	$C_8H_{14}O_4$		MS^2[173]: 59.0124(100), 113.0594 (14)	2-(1,4-Dihydroxycyclohexanyl)-acetic acid or its isomers	Organic acid
87	5.41	238.1437	238.1436	−0.71	$C_{13}H_{19}NO_3$	MS^2[238]: 138.0913(100), 120.0809(28)		9-O-Angelylretronecine	Alkaloids
88	5.51[a]	179.0349	179.0337	−7.04	$C_9H_8O_4$		MS^2[179]: 135.0437(100)	Caffeic acid	Organic acid
89	5.56	151.0400	151.0387	−1.72	$C_8H_8O_3$		MS^2[151]: 107.0487(100)	Hydroxyl benzeneacetic acid or its isomers	Organic acid
90	5.60	431.0983	431.0972	−2.64	$C_{21}H_{20}O_{10}$		MS^2[431]: 311.0552(100), 283.0603 (31)	Vitexin	Flavonoids
91	5.81	151.0400	151.0388	−0.73	$C_8H_8O_3$		MS^2[151]: 107.0487(100)	Hydroxyl benzeneacetic acid or its isomers	Organic acid
92	5.82	179.0349	179.0338	−6.26	$C_9H_8O_4$		MS^2[179]: 135.0438(100)	Caffic acid isomer	Organic acid
93	5.86	352.1754	352.1753	−0.40	$C_{18}H_{25}NO_6$	MS^2[352]: 120.0809(100), 138.0912(11)		Usaramine	Alkaloids
94	5.99	159.0662	159.0650	−7.68	$C_7H_{12}O_4$		MS^2[159]: 115.0750(100), 159.0650 (47)	3-Methyladipic acid	Organic acid
95	6.07	352.1754	352.1752	−0.57	$C_{18}H_{25}NO_6$	MS^2[352]: 138.0914(100), 120.0810(20)		Retrorsine	Alkaloids
96	6.09	334.1648	334.1648	−0.02	$C_{18}H_{23}NO_5$	MS^2[334]: 334.1647(100), 120.0808(6), 138.0915(2)		Seneciphylline	Alkaloids
97	6.27	334.1648	334.1648	−0.02	$C_{18}H_{23}NO_5$	MS^2[334]: 334.1647(100), 120.0810(5), 138.0913(2)		Seneciphylline	Alkaloids
98	6.48	370.1860	370.1859	−0.23	$C_{18}H_{27}NO_7$	MS^2[370]: 370.1866(100), 120.0813(2)		Jacoline	Alkaloids
99	6.66	354.1911	354.1905	−1.59	$C_{18}H_{27}NO_6$	MS^2[354]: 138.0915(100), 120.0811(40)		Bisline	Alkaloids
100	6.72	165.0557	165.0544	−7.92	$C_9H_{10}O_3$		MS^2[165]: 147.0438(100), 119.0488 (16)	Methyl 2-(4-hydroxyphenyl) acetate	Organic acid
101	6.83	354.1911	354.1910	−0.12	$C_{18}H_{27}NO_6$	MS^2[354]: 138.0914(66), 120.0811(34)		Bisline	Alkaloids
102	6.84	337.0928	337.0924	−1.42	$C_{16}H_{18}O_8$		MS^2[337]: 173.0443(100), 163.0388 (23)	*Cis*-4-Tran-3-O-*p*-coumaroylquinic acid	Organic acid
103	7.04	415.1034	415.1027	−1.69	$C_{21}H_{20}O_9$		MS^2 [415]: 295.0606(100), 267.0658 (37)	Puerarin	Flavonoids

(*continued on next page*)

Table 1 (*continued*)

Peak	T_r	Theoretical mass *m/z*	Experimental mass *m/z*	Error (ppm)	Formula(M)	MS/MS fragment (+)	MS/MS fragment (−)	Proposed identity	Classification
104	7.20	354.1911	354.1908	−0.63	$C_{18}H_{27}NO_6$	MS^2[354]: 138.0915(100), 120.0811(21)		Bisline	Flavonoids
105	7.25	337.0928	337.0923	−1.60	$C_{16}H_{18}O_8$		MS^2[337]: 191.0549(100), 173.0443 (28), 93.0331(17), 163.0387(12)	Tran-5-Tran-3-O-*p*-coumaroylquinic acid	Organic acid
106	7.44	240.1594	240.1593	−0.41	$C_{13}H_{21}NO_3$	MS^2[240]: 240.1593(100), 140.1069(15), 122.0966(3)		7-(2-Methylbutyryl)-retronecine	Alkaloids
107	7.64[a]	515.1195	515.1186	−1.64	$C_{25}H_{24}O_{12}$		MS^2[515]: 191.0550(100), 179.0338 (92), 135.0437(16), 353.0877(12)	1,3-Dicaffeoylquinic acid lactones	Organic acid
108	7.65[a]	163.0400	163.0388	−7.28	$C_9H_8O_3$		MS^2 [163]: 119.0488(100), 162.8379 (84), 162.8920(15)	P-Coumaric acid	Organic acid
109	7.68	183.0651	183.0652	0.57	$C_9H_{10}O_4$	MS^2[183]: 123.0442(100), 155.0702(44), 95.0496 (38), 113.9639(37), 140.0468(18)		Syringaldehyde	Organic acid
110	7.71	223.1328	223.1329	0.35	$C_{13}H_{18}O_3$	MS^2[223]: 205.1221(100)		(E,4R)-4-hydroxy-4,5,5-Trimethyl-3-(3-oxobut-1-Enyl) cyclohex-2-enone	Others
111	7.79	334.1648	334.1649	0.15	$C_{18}H_{23}NO_5$	MS^2[334]: 334.1650(100), 138.0913(91), 120.0810 (46)		Seneciphylline	Alkaloids
112	7.82	335.0772	335.0769	−0.95	$C_{16}H_{16}O_8$		MS^2 [335]: 161.0231(100), 135.0438 (25), 179.0338(14)	Caffeoyl shikimic acid	Organic acid
113	7.86	367.1034	367.1031	−0.75	$C_{17}H_{20}O_9$		MS^2[367]: 173.0443(100), 193.0495 (21), 61.9868(41)	4-Feruloylquinic acid	Organic acid
114	7.89[a]	173.0819	173.0808	−6.48	$C_8H_{14}O_4$		MS^2 [173]: 111.0801(100), 59.0124 (19)	Octanedioic acid	Organic acid
115	8.25	367.1034	367.1028	−1.67	$C_{17}H_{20}O_9$		MS^2[367]: 191.0550(100), 173.0443 (28), 93.0331(26), 193.0550(13)	5-Feruloylquinic acid	Organic acid
116	8.31	179.0349	179.0338	−6.09	$C_9H_8O_4$		MS^2[179]:135.0437(100)	Caffic acid isomer	Organic acid
117	8.37	537.1977	537.1972	−0.89	$C_{26}H_{34}O_{12}$		MS^2[537]: 327.1232(100), 165.0545 (85), 195.0650(34)	Hyuganoside III b or Citrusin A or Alaschanioside A	Others
118	8.50	197.0808	197.0808	0.28	$C_{10}H_{12}O_4$	MS^2[197]: 107.0858(100), 179.0703(40)		Cantharidin	Others
119	8.65	537.1977	537.1969	−1.47	$C_{26}H_{34}O_{12}$		MS^2[537]: 165.0544(100), 195.0653 (42), 327.1244(12)	Hyuganoside III b or Citrusin A or Alaschanioside A	Others
120	8.81	251.1277	251.1275	−1.05	$C_{14}H_{18}O_4$	MS^2[251]: 187.1116(100), 169.1011(84), 233.1117 (60), 215.1066(56)		Nor-seco-glutinosone	Others
121	8.81	338.1961	338.1959	−0.73	$C_{18}H_{27}NO_5$	MS^2[338]: 122.0961(100), 140.1065(55)		Neoplatyphylline	Alkaloids
122	8.93	367.1034	367.1031	−0.83	$C_{17}H_{20}O_9$		MS^2[367]: 179.0338(100), 135.0437 (45), 161.0231(17), 173.0442(12)	Feruloylquinic acid	Organic acid
123	9.17[a]	137.0244	137.0231	−9.61	$C_7H_6O_3$		MS^2 [137]: 93.0331(100)	Salicylic acid	Organic acid
124	9.27	338.1961	338.1958	−1.09	$C_{18}H_{27}NO_5$	MS^2[338]: 122.0966(100), 140.1070(68)		Platyphylline	Alkaloids
125	9.78	415.1034	415.1029	−1.16	$C_{21}H_{20}O_9$		MS^2 [415]: 295.0604(100), 267.0659 (29)	Puerarin isomer	Flavonoids
126	9.90	225.1485	225.1484	−0.31	$C_{13}H_{20}O_3$	MS^2[225]: 207.1379(100), 137.0961(26), 189.1273		(E)-4-((1S,3R,4R)-1-hydroxy-4,5,4-trimethyl- 7-oxabicyclo [4.1.0] heptan-1-yl)-but-1-en-3-one	Others

(*continued on next page*)

Table 1 (*continued*)

Peak	T_r	Theoretical mass *m/z*	Experimental mass *m/z*	Error (ppm)	Formula(M)	MS/MS fragment (+)	MS/MS fragment (−)	Proposed identity	Classification
						(18), 149.0961(14), 119.9605(11)			
127	9.96	463.0881	463.0879	−0.47	$C_{21}H_{20}O_{12}$		MS^2[463]: 301.0347(100)	Quercetin 5-glucoside	Flavonoids
128	10.23	225.1485	225.1485	0.173	$C_{13}H_{20}O_3$	MS^2[225]: 207.1378(100), 137.0961(27), 149.0960 (16), 189.1273(13)		(E)-4-((1S,3R,4R)-1-hydroxy-4,5,4-trimethyl-7-oxabicyclo [4.1.0] heptan-1-yl)-but-1-en-3-one	Others
129	10.33[a]	609.1461	609.1454	−1.08	$C_{27}H_{30}O_{16}$		MS^2[609]: 300.0269(100), 301.0342 (66)	Rutin	Flavonoids
130	10.36[a]	463.0881	463.0878	−0.86	$C_{21}H_{20}O_{12}$		MS^2 [463]: 300.0269(100), 301.0341 (45)	Hyperoside	Alkaloids
131	10.41	225.1485	225.1484	−0.22	$C_{13}H_{20}O_3$	MS^2[225]: 207.1379(100), 137.0961(30), 149.0962 (20), 189.1274(14)		(E)-4-((1S,3R,4R)-1-hydroxy-4,5,4-trimethyl-7-oxabicyclo [4.1.0] heptan-1-yl)-but-1-en-3-one	Others
132	10.50	415.1034	415.1026	−1.84	$C_{21}H_{20}O_9$		MS^2 [415]: 295.0604(100), 267.0663 (32)	Puerarin isomer	Flavonoids
133	10.54[a]	463.0881	463.0877	−0.92	$C_{21}H_{20}O_{12}$		MS^2 [463]: 300.0270(100), 301.0343 (50)	Isoquercitrin	Flavonoids
134	10.66[a]	449.1078	449.1083	1.05	$C_{21}H_{20}O_{11}$	MS^2 [449]: 287.0537(100), 303.0487(31), 288.0574 (13)		Luteoloside	Flavonoids
135	10.83	336.1805	336.1803	−0.47	$C_{18}H_{25}NO_5$	$MS^2$336]: 120.0810(100)		Senecionine	Alkaloids
136	10.92[a]	187.0975	187.0964	−5.89	$C_9H_{16}O_4$		MS^2 [187]: 125.0958(100)	Azelaic acid	Organic acid
137	11.03[a]	515.1195	515.1185	−1.76	$C_{25}H_{24}O_{12}$		MS^2[515]: 173.0444(100), 179.0338 (83), 191.0550(40), 135.0439(15), 353.0870(13), 161.0232(13)	3,4-Dicaffeoylquinic acid lactones	Organic acid
138	11.24	447.0932	447.0925	−1.75	$C_{21}H_{20}O_{11}$		MS^2[447]: 284.0318(100), 285.0381 (33)	Astragalin	Flavonoids
139	11.27[a]	515.1195	515.1186	−1.64	$C_{25}H_{24}O_{12}$		MS^2[515]: 191.0550(100), 179.0338 (96), 173.0443(45), 135.0437(15), 353.0872(14)	3,5-Dicaffeoylquinic acid lactones	Organic acid
140	11.42	593.1511	593.1532	3.484	$C_{27}H_{30}O_{15}$		MS^2 [593]: 285.0396(100), 284.0319 (56)	Biorobin	Flavonoids
141	11.45	269.0455	269.0449	−2.21	$C_{15}H_{10}O_5$		MS^2 [269]: 213.0545(100), 241.0492 (41), 240.0417(30), 226.0260(28)	Genistein	Flavonoids
142	11.64	447.0932	447.0928	−1.08	$C_{21}H_{20}O_{11}$		MS^2 [447]: 284.0320(100)	Kaempferol-O-β-D glucoside	Flavonoids
143	11.77	263.1288	263.1284	2.63	$C_{15}H_{20}O_4$		MS^2[263]: 204.1145(100), 219.1382 (87), 201.1274(38)	Abscisic acid isomer	Organic acid
144	12.00	477.1038	477.1040	0.37	$C_{22}H_{22}O_{12}$		MS^2[447]: 315.0504(100), 314.0424 (98), 285.0398(11)	Isorhamnetin-3-O-glucoside	Others
145	12.32	505.0987	505.0985	−0.44	$C_{23}H_{22}O_{13}$		MS^2[505]: 300.0271(100), 301.0350 (34)	Quercetin 3-(6-O-acetyl-beta-glucoside)	Flavonoids
146	12.40	499.1245	499.1239	−1.37	$C_{25}H_{24}O_{11}$		MS^2[499]: 173.0443(100), 163.0388 (51), 179.0342(11)	*Cis*-3-*p*-coumaroyl-5-caffeoylquinic acids	Organic acid
147	12.44[a]	515.1195	515.1186	−1.64	$C_{25}H_{24}O_{12}$		MS^2[515]: 173.0443(100), 179.0338 (81), 191.0550(29), 353.0871(17), 135.0437(13)	4,5-Dicaffeoylquinic acid lactones	Organic acid
148	12.57	373.1281	373.1280	−0.34	$C_{20}H_{20}O_7$	MS^2 [373]: 327.1718(100)		Isosinensetin	Flavonoids

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Table 1 (*continued*)

Peak	T_r	Theoretical mass *m/z*	Experimental mass *m/z*	Error (ppm)	Formula(M)	MS/MS fragment (+)	MS/MS fragment (−)	Proposed identity	Classification
149	12.76[a]	263.1288	263.1283	2.06	$C_{15}H_{20}O_4$		MS^2 [263]: 219.1379(100), 204.1147 (70), 151.0752(67), 201.1271(35), 152.0833(35)	Abscisic acid	Organic acid
150	13.20	265.1445	265.1439	−2.31	$C_{15}H_{22}O_4$		MS^2 [265]: 265.1438(100), 203.1433 (78), 221.1535(21), 201.1276(10)	Mairetolide F or its isomers	Others
151	13.38	497.1089	497.1088	−0.21	$C_{25}H_{22}O_{11}$		MS^2[497]: 161.0232(100), 335.0768 (62), 179.0336(32), 137.0231(28), 135.0440(23)	Dicaffeoylquinic acid lactones	Organic acid
152	13.39	499.1245	499.1225	−4.17	$C_{25}H_{24}O_{11}$		MS^2[499]: 173.0445(100), 179.0339 (27), 191.0547(20), 59.0123(12)	4C,5p-coumaroylcaffeoylquinic acids	Organic acid
153	13.45[a]	301.0353	301.0349	−1.34	C15H10O7		MS2[301]: 151.0022(100), 178.9973 (49)	Quercetin	Flavonoids
154	13.67	269.0455	269.0452	−1.28	$C_{15}H_{10}O_5$		MS^2 [269]: 241.0497(100), 213.0547 (18), 195.0440(18), 240.0419(16), 224.0470(14)	Galangin	Flavonoids
155	13.68	497.1089	497.1076	−2.60	$C_{25}H_{22}O_{11}$		MS^2[497]: 179.0338(100), 161.0231 (90), 335.0769(71), 135.0436(44), 137.0229(17)	Caffeoylquinic acid-4'-hexoside	Others
156	14.33	253.0506	253.0502	−1.70	$C_{15}H_{10}O_4$		MS^2[253]: 225.0551(100), 209.1175 (84)	7,8-Dihydroxyflavone	Flavonoids
157	14.77	267.0662	267.0660	−1.01	$C_{16}H_{12}O_4$		MS^2[267]: 223.1344(100), 252.0423 (77)	Formononetin	Flavonoids
158	14.81	229.1445	229.1438	−3.02	$C_{12}H_{22}O_4$		MS^2[229]: 211.1330(100), 167.1429 (59)	Dodecanedioic acid	Organic acid
159	15.86	253.0506	253.0502	−1.35	$C_{15}H_{10}O_4$		MS^2[253]: 209.1535(100)	Rubiadin	Others
160	15.91	293.1758	293.1752	−1.85	$C_{17}H_{26}O_4$		MS^2[293]: 221.1537(100), 236.1046 (98), 220.1459(37)	6-Gingerol	Organic acid
161	16.79	593.1300	593.1294	−1.01	$C_{30}H_{26}O_{13}$		MS^2[593]: 121.0280(100), 209.0444 (55)	Procyanidin	Others
162	16.92	373.1281	373.1277	−1.23	$C_{20}H_{20}O_7$	MS^2 [373]: 358.1039(100)		Tangeretin	Flavonoids

[a] Indicates comparison with a standard.

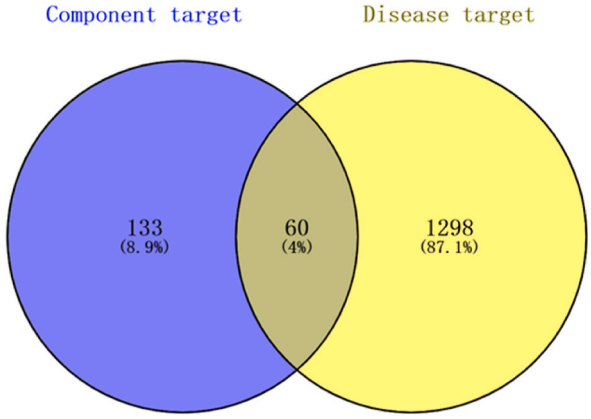

Fig. 2. Venny diagram of drug–disease common targets.

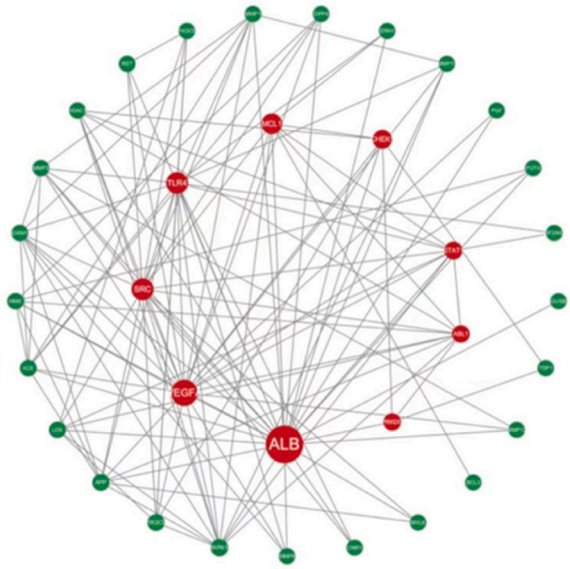

Fig. 3. PPI network.

mass fragmentation pathways, which is fragmentation ions at *m/z* 179.0339 and 135.0437 through the loss of the saccharide moiety 162 Da and saccharide moiety and CO_2 moiety 206 Da. Therefore, each compound was assigned as caffeoyl hexoside [14].

Compounds 31, 42, 54, 62, 74, 79, and 82 showed the same quasi-molecular ion at *m/z* 515.1406 and fragmentation ion at *m/z* 191.0547 and 179.0337, suggesting the presence of a caffeoylquinic acid and hexoside moiety. Thus, they were tentatively identified as eaffeoylquinic acid-hexoside. Similarly, the other chlorogenic acid derivatives were identified [14].

3.1.3. Flavonoids

Compounds 103, 125, and 132 showed retention times of 7.04, 9.78, and 10.50 min, with the precursor at *m/z* 415.1034 $[M-H]^-$, yielding indicative fragment ions *m/z* 295.0606 and *m/z* 267.0658. Thus, compounds 103, 125, and 132 were identified as purarin isomers.

Compound 127 showed a retention time of 9.96 min and a quasi-molecular ion at *m/z* 463.0881 $[M-H]^-$, yielding *m/z* 301.0347; it was identified as quercetin 5-glucoside [15]. Similarly, compounds 140 and 145 were identified biorobin and quercetin 3-(6-O-acetyl -beta-glucoside), respectively [13].

Compounds 90, 138, 141, 142, 148, 154, 156, 157, and 162 were identified as vitexin, astragalin, genistein, kaempferol-o-β-D-glucoside, isosinensetin, galangin, 7,8-dihydroxyflavone, formononetin, and tangeretin, respectively, by comparison with the OTCML databases.

3.1.4. Alkaloids

Compounds 96, 97, and 111 showed the quasi-molecular ion at *m/z* 334.1648 (0 ppm, $C_{18}H_{24}NO_5$), 334.1648 (0 ppm, $C_{18}H_{24}NO_5$),

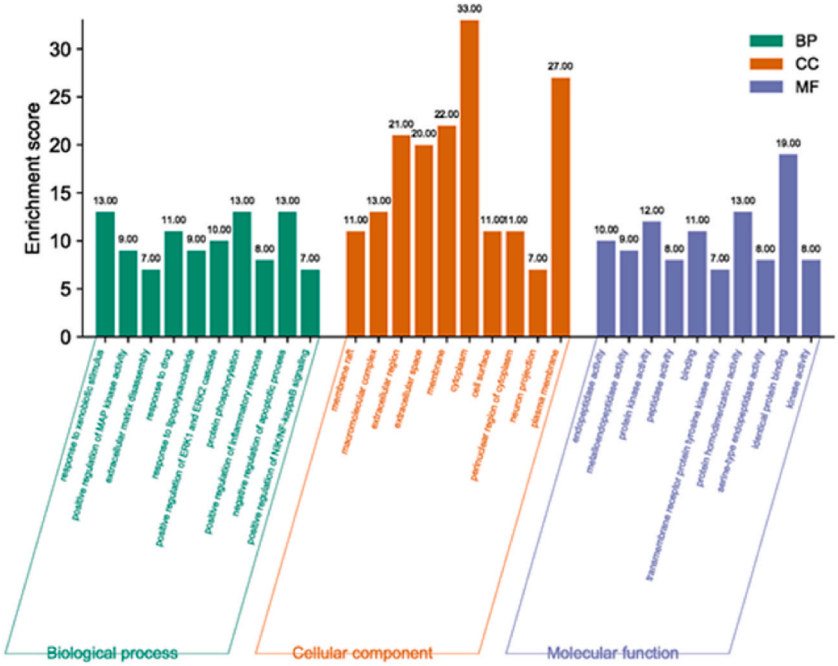

Fig. 4. Go pathway enrichment analysis.

and 334.1650 (0.15 ppm, $C_{18}H_{24}NO_5$), and generated fragment ions at *m/z* 138.09 and 120.08, which were the diagnostic ions for retronecine-type pyrrolizidine alkaloids. Thus, they were tentatively identified as seneciphylline isomers. Similarly, 9-O-angelylretronecine(87), 7-angeloylretronecine(77), 7-angeloylretronecine N-oxide(16, 32), creatonotine(73), seneciphylline(96, 97, 111), Senecionine(135), Riddelline(60), Integerrimine N-oxide(58), Jacobine(81), usaramine(93), retrorsine(95), bisline(99, 101, 104),

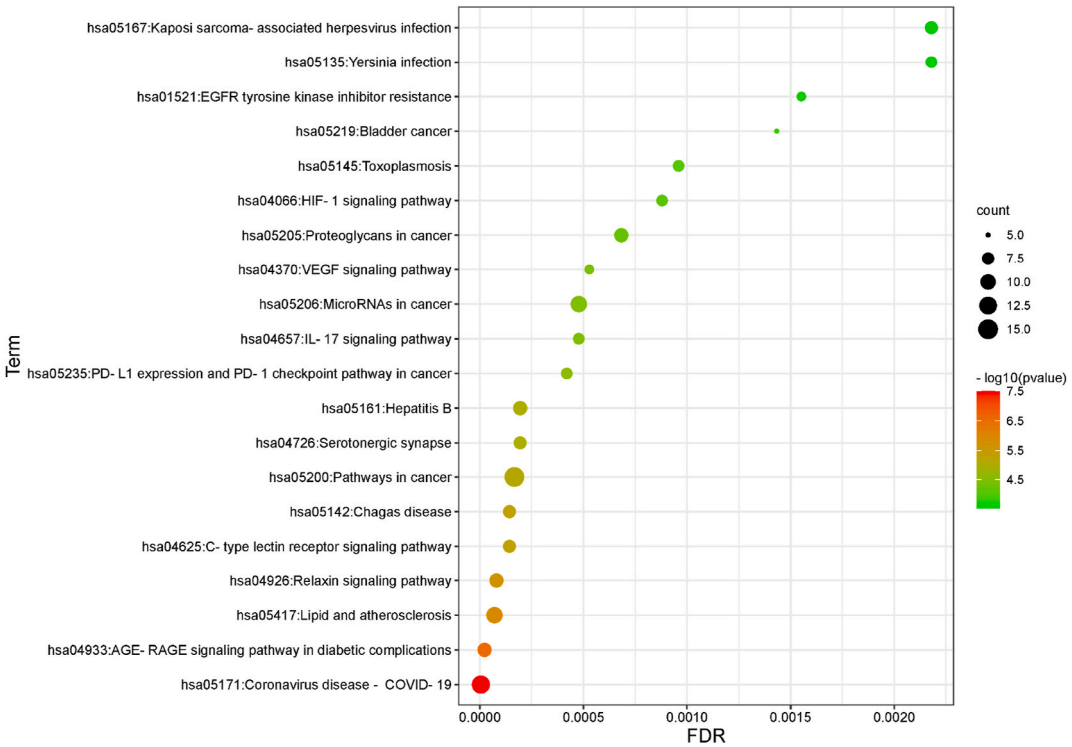

Fig. 5. KEGG pathway analysis.

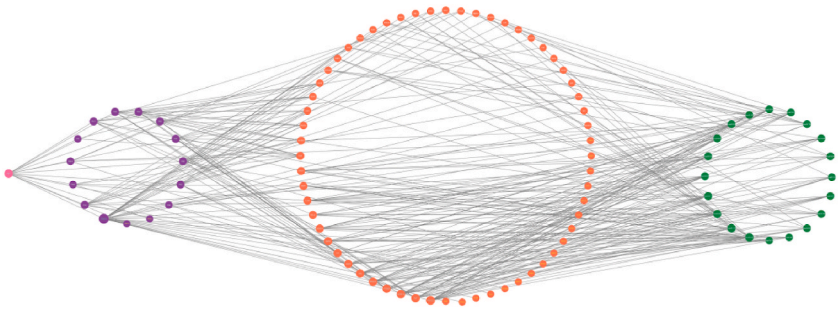

Fig. 6. Drug–component–target–pathway network.

riddelline N-oxide(38), retrorsine N-oxide(35), jacoline(20, 98), and jaconine(66) were identified [16–22]. Conversely, compounds 106, 27, 121, and 124 showed the quasi-molecular ion at *m/z* 240.1594 (0.41 ppm, $C_{13}H_{21}NO_3$), 254.1386 (0 ppm, $C_{13}H_{19}NO_4$), 338.1961 (0.73 ppm, $C_{18}H_{27}NO_5$), and 338.1961 (1.09 ppm, $C_{18}H_{27}NO_5$), respectively, and generated the fragment ions at *m/z* 122.09 and 140.10, which were the diagnostic ions for platynecine-type pyrrolizidine alkaloids. Thus, they were tentatively identified as 7-(2-methylbutyryl)-retronecine (106), 9-angeloylretronecine N-oxide (27), neoplatyphylline (121), and platyphylline (124), respectively, which are characteristic of platynecine-type PAs [21,22].

3.1.5. Others

Two isomers, 117 and 119, showed the molecular ions at *m/z* 537.1973. Based on the MS and MS^2 spectra and the literature [23], the isomers were characterized as hyuganoside III b citrusin A or alaschanioside A. Compounds 18, 21, 30, 47, 56, 71, 80, 110, 120, 150, and 161 were tentatively identified as tetrahydro-jacaranone acid, tetrahydroxyxanthone, 2-(3,4-dihydroxyphenyl)ethyl alcoho, cannabiside C, esculin hydrate, coumaroylhexos [23], esculetin [13], (E,4R)-4-hydroxy-4,5,5-trimethyl-3-(3-oxobut-1-enyl)cyclo-hex-2-enone, Nor-seco-glutinosone, mairetolide F or its isomers. and procyanidin, respectively [17]. Compounds 1, 2, 9, 118, and 159 were identified as mannitol, lactose, uridine, cantharidin, and rubiadin, respectively, by comparing them with the databases.

3.2. Network pharmacology

3.2.1. Screening of effective components of Feining Granules

Overall, 15 chemicals were selected as active chemicals by OB and DL, and 193 targets of Feining Granules were obtained using the TCMSP and Swiss Target Prediction databases.

3.2.2. Screening of drug–disease common targets

In total, 1358 potential targets of chronic bronchitis were obtained by searching for the keyword “chronic bronchitis” and removing repeated targets. Sixty drug–disease common targets were obtained using the Venny2.1.0 platform (Fig. 2).

3.2.3. PPI network

Cytoscape developed a PPI network with 34 nodes and 129 edges (Fig. 3). The core targets of the Feining Granules in treating chronic bronchitis were selected as ALB, VEGFA, SRC, TLR4, MAPK14, MCL1 and STAT1 according to the top nine-degree values.

3.2.4. KEGG pathway analysis and GO enrichment analysis of targets of feining granules

GO enrichment and KEGG pathway analyses were performed on the DAVID database by uploading the 60 common targets. Finally, GO enrichment analysis resulted in 430 GO items, most associated with inflammation: regulation of the immune system, signal transduction, apoptosis, and other biological processes. Fig. 4 presents the top 10 GO entries with significant enrichment of common targets in biological processes, cell composition, and molecular functions. Overall, 105 pathways were obtained ($P < 0.05$), including the HIF-1 signaling pathway, VEGF signaling pathway, IL-17 signaling pathway, and other signaling pathways. The top 20 KEGG pathways that were significantly enriched were identified (Fig. 5).

3.2.5. Drug–component–target–pathway network

Cytoscape 3.9.0 software was used to establish the “drug-component–target–pathway” network model. Overall, 96 nodes (1 drug node, 15 component nodes, 60 target nodes, and 20 pathway nodes) were identified (Fig. 6). Hence, the “drug–component–target–pathway” network of the Feining Granules has one molecule acting on multiple targets and multiple targets interacting with one molecule, which preliminarily explains the drug material basis and action mechanism of the Feining Granules in treating chronic bronchitis.

4. Conclusion

This study developed a systematic method for characterizing the chemical and pharmacological mechanisms of Feining Granules

for the treatment of chronic bronchitis based on the UHPLC–Q-exactive orbitrap MS and network pharmacology. Overall, 162 chemicals were characterized and identified; 99 were reported for the first time to the best of our knowledge. Overall, 15 key active ingredients, including vitexin, puerarin, and hirsutrin, were characterized and associated with the *ALB*, *VEGFA*, and *SRC* target genes according to their influence on the HIF-1, VEGF, and other signal pathways. These results provide information to understand the effective substances of Feining Granules and improve quality control.

Ethics declarations

Review and approval by the ethics committee was not needed for this study because it did not involve animal or human clinical trials and was not unethical.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Jiaxin Li: Writing – original draft, Data curation. **Yuqi Chen:** Writing – original draft, Data curation. **Kaiquan Yu:** Data curation. **Min Zhang:** Data curation. **Qing Li:** Data curation. **Sunv Tang:** Data curation. **Yanlan Liu:** Data curation. **Hui Li:** Writing – review & editing. **Zaiqi Zhang:** Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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