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Rapid characterization of chemical constituents of Shaoyao Gancao decoction using UHPLC coupled with Fourier transform ion cyclotron resonance mass spectrometry†

Lin Sun,‡[a] Min Zhao,‡[a] Yanhui Zhao,[a] Xue Jiang,[a] Miao Wang,*[b] Yixin Zhang[a] and Chunjie Zhao*[a]

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Shaoyao Gancao decoction (SGD), a well-known Chinese herbal formula, has been used to treat liver injury for a long time. In this study, chemical profiles of SGD were identified using ultra high-performance liquid chromatography combined with Fourier transform ion cyclotron resonance mass spectrometry (UHPLC-FT-ICR-MS/MS). Liquid chromatography was performed on a C_{18} column (150 mm × 2.1 mm, 1.8 μm); the mobile phase comprised 0.1% formic acid (A) and acetonitrile (B). We then characterized 73 chemical compounds; the primary constituents in SGD included phenols and monoterpenes (in Paeoniae Radix Alba), triterpene saponins, and flavonoids (in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle). Thus, this study provides a basis for further study on SGD and is expected to be useful for rapidly characterizing constituents in other traditional Chinese herbal formulations.

1 Introduction

In China, Traditional Chinese Medicine (TCM) and its formulas have an extended history for treating diseases. Their integrated and synergistic effects on multiple targets have been extensively praised.[1] However, it is difficult for researchers to explain the component that plays a major role in the efficacy of the materials because of their massive chemical composition, which is an obstacle for TCM in the international market.[2] In reaction to this phenomenon, multiple studies have focused on examining the chemical components of TCM. However, TCM is numerous in quantity and complicated in composition, so the condition of current research is far from enough. Because of the continuous development in science and technology, a rapid method for identifying chemical components of TCM is necessary, which will then act as the basis for TCM's pharmacology research and clinical applications.

Initially, Shaoyao Gancao decoction (SGD) was described in Shang Han Lun, a clinical TCM book written by Zhang Zhongjing in the Eastern Han Dynasty.[3] It contains two herbs: Paeoniae Radix Alba and Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle. The SGD was a classical formula of TCM and extensively used for treating febrile diseases such as relief of nourishing liver, relaxing spasm, and relieving pain.[4]

At present, few studies have focused on the chemical components of SGD.[5] To improve the detection range and sensitivity of previous method, researchers are increasingly using UHPLC-FT-ICR-MS, which is a type of powerful qualitative screening platform with a high mass resolving power that demonstrates powerful separation and can generate accurate molecular measurements. For example, using this method, Wang *et al.* characterized 33 chemical compounds in Cortex Fraxini and Guan *et al.* characterized 120 chemical compounds in Sijunzi decoction.[6,7]

In our work, we selected UHPLC-FT-ICR-MS to systematically characterize the chemical profiles of SGD. This study is thus able to provide a substantial base and provide considerable information for SGD-related pharmacological research.

2 Materials and methods

2.1 Chemicals and materials

Paeoniae Radix Alba (batch number: 18061201, source: Anhui China) and Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle (batch number: 180518; source: Neimenggu China), which were authenticated by Professor Jingming Jia (Department of TCM, Shenyang Pharmaceutical University, Shenyang, China), were purchased from Guoda pharmacy (Shenyang, China). The primary source of reference compounds (purity > 98%), including benzoyl paeoniflorin, albiflorin, ononin, and glycyrrhizic acid, was Shanghai Yuanye Bio-Technology Co., Ltd

[a]*School of Pharmacy, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang, Liaoning Province, China. E-mail: lab433@163.com*

[b]*School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang, Liaoning Province, China*

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‡ Co-first authors.

(Shanghai, China), while gallic acid, liquiritin, paeoniflorin, and liquiritin apioside were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Moreover, acetonitrile of HPLC grade and formic acid of LC-MS grade were obtained from Fisher Scientific (Fair Lawn, NJ, USA); purified water was then purchased from Wahaha (Hangzhou, China).

2.2 Preparation of SGD for analysis

As per SGD's original composition, two constituting herbs, Paeoniae Radix Alba (250 g) and Glycyrrhizae Radix et Rhizoma Praeparata (250 g), were mixed and macerated in purified water (5 L) for 0.5 h, then boiled at 100 °C for 1.5 h, and then the extracted solution was filtered through five layer gauzes. The residue was decocted twice with boiling water (1 : 8, v/v) for 1 h each and the extracted solution was filtered using five layer gauzes. These three extractions were then combined and dried using lyophilization. Before analysis, dried powder (0.5 g) was dissolved in water (10 mL), and then vortexed for 1 min for complete dissolution.

2.3 Instrument and analytical conditions

Chromatographic analysis was performed using an Agilent 1260 UHPLC system (USA) using a universal XB C_{18} column (150 mm × 2.1 mm, 1.8 μm; Kromat, USA) at the column temperature of 35 °C. The mobile phase comprised 0.1% formic acid (A) and acetonitrile (B), and the gradient elution program was carried out for chromatographic separation as follows: 2–10% (B) from 0 to 12 min, 10–25% (B) from 12 to 32 min, 25–62% (B) from 32 to 52 min, and 62–65% (B) from 52 to 55 min. The flow rate was 0.20 mL min^{-1}, and the injection volume was 2 μL.

Mass spectra analysis was conducted on a Bruker Solarix 7.0 T FT-ICR-MS system (Bruker, Germany) and a Bruker Compass-Hystar workstation (Bruker, Germany) using both positive and negative electrospray ionization (ESI) modes, followed by optimized conditions: nebulizer gas pressure of 4.0 bar; dry gas flow rate of 8 L min^{-1}; dry gas temperature of 200 °C; ion accumulation time of 0.15 s; time of flight of 0.6 ms; capillary voltage of 4.5 kV; and endplate offset of 500 V. The recording of the full-scan mass spectrum data was performed between m/z 100 and 3000. In respect to the auto MS/MS mode, the selection of both MS/MS boost and MS/MS isolation was made; moreover, the range of collision power was maintained between 10 and 30 eV for MS/MS experimentations.

3 Results and discussion

Fig. 1 shows the base peak ion chromatograms (BPC) of SGD and the reference compounds. The extracted ion chromatograms (EIC) for each molecular weight, which are shown in the ESI (Fig. S1 and S2),† were correspondingly obtained for detecting the associated compound. Among the identified compounds, the accurate identification of eight compounds was performed by comparing the retention time (t_R) and the MS/MS data associated with the reference compounds in the positive ion mode. The other compounds were determined by their retention times, as well as the molecular weight and the MS/MS fragments. Bruker workstation was used for computing the molecular formulas of the compounds by comparing the known molecular weights with the measured molecular weights, followed by limiting the acceptable error values to <3.0 ppm. Using MS/MS data, additional speculations of the layouts of the compounds were conducted. In aggregate, we reported 73 compounds and their

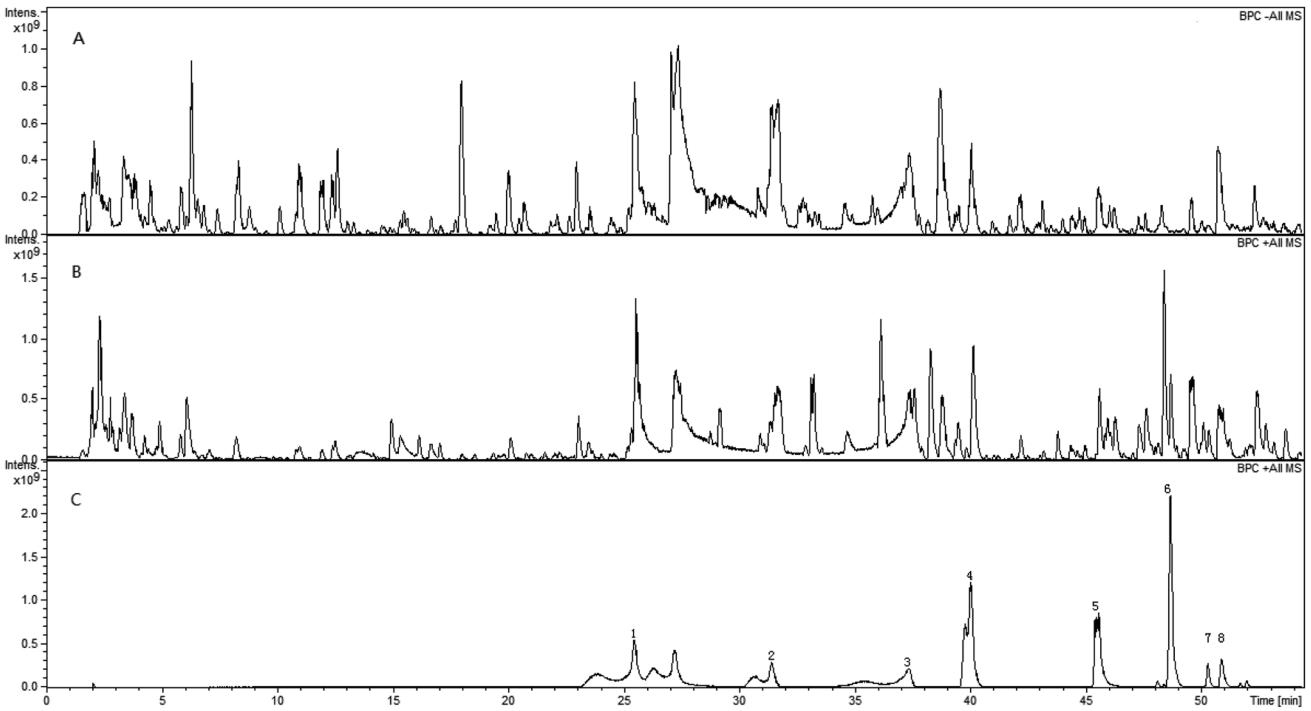

Fig. 1 The base peak ion chromatograms (BPC) of SGD in both positive (A) and negative (B) ion modes and the corresponding compounds (C).

layouts are presented in Fig. S3 and S4.† Fig. S5† shows the presentation of MS/MS spectra of the typical compounds while displaying their possible fragmentation pathways in Fig. 2. The inferences of each ingredient were carried out with the help of the molecular formulas and fragmentation pathways, followed by additional confirmation with reference to the previous literatures.[8–16] Table 1 lists the retention time, formula, molecular weight, calculated *m*/*z*, detected *m*/*z*, error value and MS/MS data

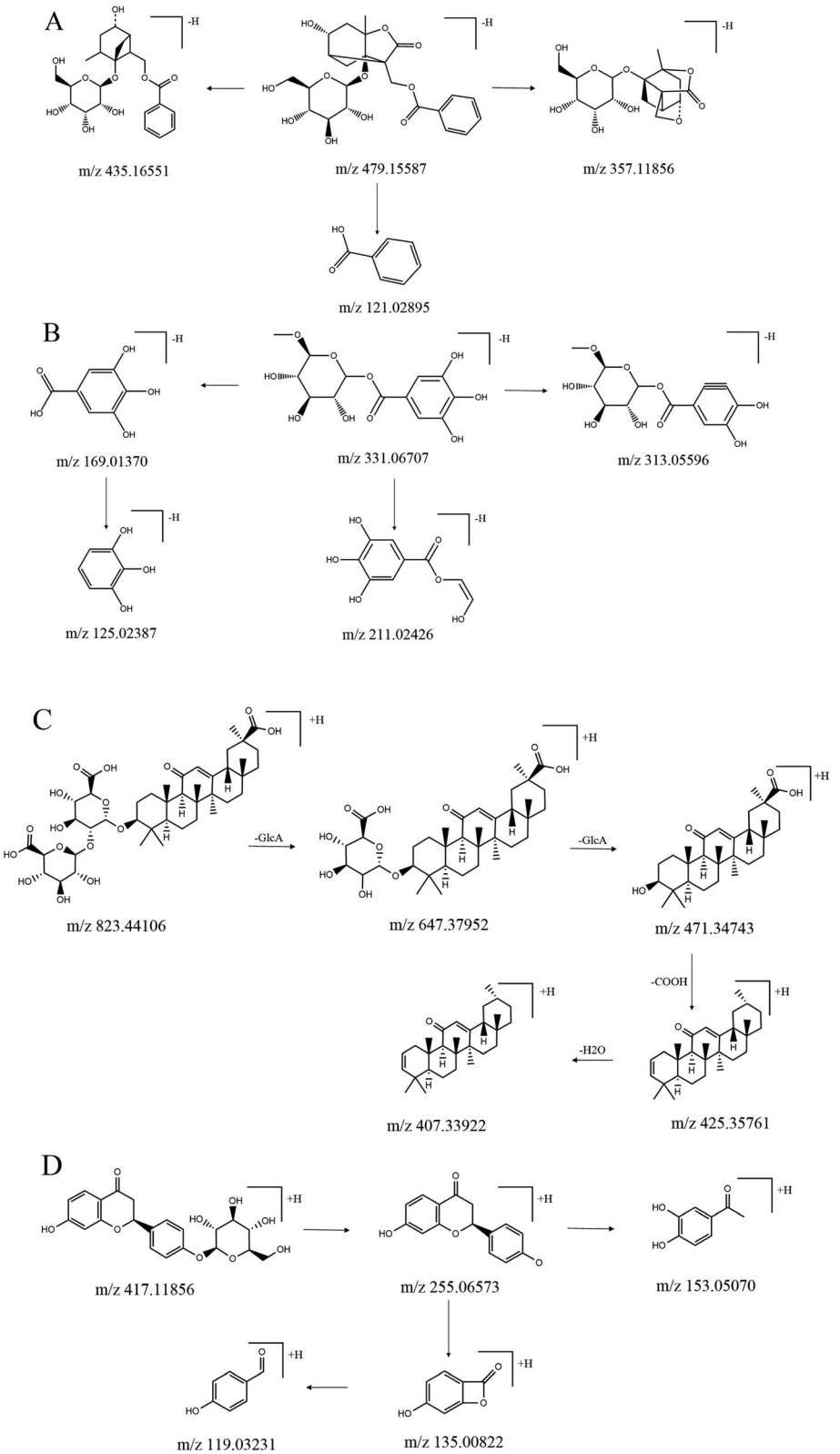

Fig. 2 The possible fragmentation pathways of the typical compounds. (A) Albiflorin, (B) glucogallin, (C) glycyrrhizic acid, (D) liquiritin.

Table 1 UHPLC-FT-ICR-MS analysis of Shaoyao Gancao detection[a]

No.	t_R (min)	Identification	Formula	Molecular weight	Ion mode	MS (m/z)	ppm	MS/MS (m/z)
1	3.43	Citric acid	$C_6H_8O_7$	192.0270	$[M + H]^+$	193.03428	−0.55	191.05401; 111.00913
					$[M - H]^-$	191.01973	0.44	133.01330
2	6.2	Gallic acid	$C_7H_6O_5$	170.0215	$[M + H]^+$	171.0288	5.15	126.02387
					$[M - H]^-$	169.01425	0.54	125.02453; 108.02271
3	6.72	Debenzoyl paeoniflorin	$C_{16}H_{24}O_{10}$	376.1369	$[M + H]^+$	377.14422	1.62	375.12803; 345.11810; 195.06531; 139.07233
4	7.43	1-*O*-β-D-Glucopyranosyl-paeonisuffrone	$C_{16}H_{24}O_9$	360.1420	$[M + H]^+$	361.14931	1.49	181.08418; 163.01784; 127.01413
5	11.93	Glucogallin	$C_{13}H_{16}O_{10}$	332.0743	$[M + H]^+$	333.08162	1.37	207.05048; 125.02387
					$[M - H]^-$	331.06707	−0.11	313.05596; 211.02426; 169.01370; 125.02387
6	12.97	6-*O*-β-D-Glucopyranosyl lactinolide	$C_{16}H_{26}O_9$	362.1576	$[M - H]^-$	361.15041	0.28	185.11777; 163.06065; 113.06025
7	15.66	Ethyl gallic acid	$C_9H_{10}O_5$	198.0528	$[M + H]^+$	199.0601	3.32	125.10300
8	17.64	Mudanpioside F	$C_{16}H_{24}O_8$	344.1471	$[M - H]^-$	343.13981	1.1	179.05556; 165.09115
9	24.29	Galloylpaeoniflorin	$C_{30}H_{32}O_{15}$	632.1741	$[M + H]^+$	633.1814	1.00	631.16613; 613.15570; 509.12952; 491.11895; 463.12404
10	24.48	1′-*O*-Benzoylsucrose	$C_{19}H_{26}O_{12}$	446.1424	$[M - H]^-$	445.13515	0.07	179.14800; 132.04226; 121.02895
11	24.87	Isomaltopaeoniflorin	$C_{29}H_{38}O_{16}$	642.2159	$[M + H]^+$	643.22326	1.49	643.22326; 191.11500
12	25.22	Paeonol	$C_9H_{10}O_3$	166.0630	$[M - H]^-$	165.05572	0.59	165.05572
13	25.54	Paeonilactone B	$C_{10}H_{12}O_4$	196.0735	$[M + H]^+$	197.08084	0.43	133.0662; 105.0688; 103.0545
14	25.56	Paeonilactone C	$C_{17}H_{18}O_6$	318.1103	$[M + H]^+$	319.11761	0.34	183.06573; 135.04460
15	26.92	Oxypaeoniflorin	$C_{23}H_{28}O_{12}$	496.1581	$[M + H]^+$	497.16535	1.07	267.08286; 180.07864; 163.06065; 137.02837
16	28.75	Schaftoside	$C_{26}H_{28}O_{14}$	564.1479	$[M + H]^+$	565.15518	1.41	565.15518; 501.13969; 163.03952
					$[M - H]^-$	563.14063	1.16	563.14063; 499.1404
17	31.1	Palmitic acid	$C_{16}H_{32}O_2$	256.2402	$[M + H]^+$	257.08084	1.19	143.07120; 113.11303
18	31.33	Paeoniflorigenone	$C_{17}H_{18}O_6$	318.1103	$[M + H]^+$	319.11761	1.29	137.05818; 133.06662; 105.03324
19	31.35	Albiflorin	$C_{23}H_{28}O_{11}$	480.1631	$[M + H]^+$	481.17044	0.53	197.08113; 151.07255; 133.02649; 105.01342
					$[M - H]^-$	479.15587	0.67	435.16551; 357.11856; 121.02895
20	31.85	Kaempferitrin	$C_{27}H_{30}O_{14}$	578.1635	$[M + H]^+$	579.17083	0.49	623.15923; 315.05121; 314.04118; 299.01050
21	38.83	Lactiflorin	$C_{23}H_{26}O_{10}$	462.1526	$[M + H]^+$	463.15987	1.59	179.07100; 151.07186; 135.08121
					$[M - H]^-$	461.14532	0.45	461.14532; 285.06104; 121.08956
22	37.45	Paeonisuffrone C	$C_{10}H_{14}O_4$	198.0892	$[M - H]^-$	197.08084	0.6	197.08084
23	37.53	Paeoniflorin	$C_{23}H_{28}O_{11}$	480.1631	$[M + H]^+$	481.17044	0.8	481.17044; 451.16042; 375.12972; 329.12364; 123.04460
24	40.58	Benzoyloxypaeoniflorin	$C_{30}H_{32}O_{13}$	600.1842	$[M - H]^-$	599.17701	0.7	599.17618; 509.19525; 491.23538; 293.21011; 137.10284
25	40.59	Mudanpioside D	$C_{24}H_{30}O_{12}$	510.1737	$[M - H]^-$	509.16645	0.81	509.16645; 463.15486; 121.02994
26	44.4	Hederagenin	$C_{30}H_{48}O_4$	472.3553	$[M + H]^+$	473.36254	1.45	426.31340; 251.20111; 168.11503
27	46.06	Benzoylpaeoniflorin	$C_{30}H_{32}O_{12}$	584.1893	$[M + H]^+$	585.19665	1.33	585.19665; 463.16042; 433.14986;
28	49.25	Benzoyl paeonifloride	$C_{30}H_{32}O_{12}$	584.1893	$[M + H]^+$	585.19665	0.99	585.19665; 567.18664; 463.45900
29	50.79	Astrantiagenin D	$C_{30}H_{46}O_4$	470.3396	$[M + H]^+$	471.34689	1.04	234.16198; 209.45415
30	52.15	Oleanolic acid	$C_{30}H_{48}O_3$	456.3604	$[M + H]^+$	457.36762	1.17	411.28992; 203.16068; 153.15942;

Table 1 (*Contd.*)

No.	t_R (min)	Identification	Formula	Molecular weight	Ion mode	MS (m/z)	ppm	MS/MS (m/z)
31	2.01	Gentiobiose	$C_{12}H_{22}O_{11}$	342.1161	$[M-H]^-$	341.10894	0.29	341.10894; 221.06613; 179.05556; 161.04500
32	23.43	Liquiritigenin-7,4-diglucoside	$C_{27}H_{32}O_{14}$	580.1791	$[M-H]^-$	579.17193	1.66	579.17193; 417.11856; 253.05008
33	23.59	Liquiritin	$C_{21}H_{22}O_9$	418.1263	$[M-H]^-$	417.11911	0.26	255.06573; 153.05070; 135.00822; 119.03231
34	26.07	Vicenin-2	$C_{27}H_{30}O_{15}$	594.1584	$[M+H]^+$	595.16575	0.85	595.16575; 451.14517
	25.97				$[M-H]^-$	593.15119	1.31	593.15119; 449.12952; 363.12912
35	28.75	Schaftoside	$C_{26}H_{28}O_{14}$	564.1479	$[M+H]^+$	565.15518	1.41	446.11564; 431.10298; 401.09589
					$[M-H]^-$	563.14063	1.16	403.10291; 271.05008
36	30.22	Choerospondin	$C_{21}H_{22}O_{10}$	434.1213	$[M-H]^-$	433.11249	−0.12	282.11643; 271.06593; 152.01479
37	31.7	Pinocembrin	$C_{15}H_{12}O_4$	256.0735	$[M+H]^+$	257.08084	1.96	257.08084; 108.02113
					$[M-H]^-$	255.06628	0.16	255.06628; 150.03169; 106.04186
38	30.88	Glucoliquiritin apioside	$C_{32}H_{40}O_{18}$	712.2214	$[M+H]^+$	713.22874	0.94	551.17647; 459.70586; 255.13625
39	31.12	Licoagroside A	$C_{23}H_{24}O_{12}$	492.1268	$[M-H]^-$	491.11950	0.88	327.07693; 164.03532; 148.03643
40	31.66	Liquiritin apioside	$C_{26}H_{30}O_{13}$	550.1686	$[M+H]^+$	551.17592	0.92	551.17592; 257.09195; 137.02387
					$[M-H]^-$	549.16136	1.24	549.16136; 417.17138; 255.13727; 135.00822
41	30.95	Liquiritigenin	$C_{15}H_{12}O_4$	256.0735	$[M+H]^+$	257.08084	1.19	257.08084; 135.09800
42	31.10	Isoliquiritigenin	$C_{15}H_{12}O_4$	256.0735	$[M+H]^+$	257.08084	1.19	163.06592; 150.03169; 106.12400
43	31.24	Trifolirhizin	$C_{22}H_{22}O_{10}$	446.1213	$[M+H]^+$	447.12857	1.15	285.07128; 229.08474; 149.02177
44	31.53	Neoliquiritin	$C_{21}H_{22}O_9$	418.1263	$[M+H]^+$	419.13366	0.33	419.13366; 257.08138
					$[M-H]^-$	417.1186	0.13	417.11856; 255.06573
45	31.85	Violanthin	$C_{27}H_{30}O_{14}$	578.1635	$[M+H]^+$	579.17083	0.49	579.17083; 549.16082; 495.12912
46	36.34	Naringenin-7-*O*-glucoside	$C_{21}H_{22}O_{10}$	434.1212	$[M-H]^-$	433.11402	1.9	433.11402; 271.06065
47	37.53	Albiflorin	$C_{23}H_{28}O_{11}$	480.1631	$[M+H]^+$	481.17044	0.82	481.17044; 451.44800; 359.31451; 329.12364
48	40.13	Ononin	$C_{22}H_{22}O_9$	430.1264	$[M+H]^+$	431.13366	0.46	323.07669; 179.05556; 144.02113; 107.04969
49	40.16	Pallidiflorin	$C_{16}H_{12}O_4$	268.0735	$[M+H]^+$	269.08084	0.66	269.08084; 254.05791; 241.05008; 181.06534
					$[M-H]^-$	267.06628	0.22	267.06628; 252.04226; 223.03952
50	40.25	Isoliquiritin apioside	$C_{26}H_{30}O_{13}$	550.1686	$[M+H]^+$	551.17592	0.92	419.13421; 255.06572; 137.04460
					$[M-H]^-$	549.16136	1.24	549.16082; 431.11895; 415.16042
51	40.98	5,7-Dihydroxyflavone	$C_{15}H_{12}O_4$	256.0735	$[M-H]^-$	255.06628	0.46	255.06628; 135.03954; 119.04960
52	41.06	Licochalcone B	$C_{16}H_{14}O_5$	286.08412	$[M-H]^-$	285.07685	0.15	255.07891; 193.05761; 165.06538
53	43.22	Licorice-saponin O4	$C_{54}H_{84}O_{24}$	1116.5352	$[M+H]^+$	1117.5425	0.57	516.34509; 327.32421; 192.02700; 189.16433
54	44.05	Echinatin	$C_{16}H_{14}O_4$	270.08921	$[M+H]^+$	271.09649	1.14	239.07549; 149.06349; 121.03782
55	44.23	Uralsaponin T	$C_{48}H_{74}O_{19}$	954.48240	$[M+H]^+$	955.48899	0.75	779.44623; 458.35522; 179.04616
56	44.46	Uralsaponin P	$C_{42}H_{64}O_{16}$	824.41944	$[M+H]^+$	825.42671	1.04	663.36548; 487.33574; 165.06255
57	45.46	Licorice-saponin M3	$C_{48}H_{74}O_{19}$	954.4824	$[M+H]^+$	955.48971	1.32	955.48971; 517.23599; 366.04062; 163.06065

Table 1 (*Contd.*)

No.	t_R (min)	Identification	Formula	Molecular weight	Ion mode	MS (*m*/*z*)	ppm	MS/MS (*m*/*z*)
58	45.84	Uralsaponin F	$C_{44}H_{64}O_{19}$	896.4041	$[M + H]^+$	897.41146	0.7	721.14563; 545.33269; 527.88076; 467.33254; 421.11257; 497.88210; 375.33245
					$[M - H]^-$	895.3969	1.81	719.36098; 543.11527; 525.35432; 465.88908; 419.44671; 495.54490; 373.32157
59	47.3	22-Acetoxyl-glycyrrhizin	$C_{44}H_{64}O_{18}$	880.4092	$[M + H]^+$	881.4165	1.53	705.13564; 529.11253; 518.00490; 451.33235; 405.44267
60	47.6	Licorice-saponin G2	$C_{42}H_{62}O_{17}$	838.3986	$[M + H]^+$	839.40598	0.57	839.40598; 663.35370; 487.37913
					$[M - H]^-$	837.39142	0.6	837.39142; 661.12531; 485.90786; 351.11236
61	48.42	Licorice-saponin A3	$C_{48}H_{72}O_{21}$	984.4565	$[M + H]^+$	985.46389	0.23	985.46389; 823.88097; 647.32446
					$[M - H]^-$	983.44933	0.3	983.44933; 821.57765; 645.33542; 351.11676
62	48.49	Uralsaponin N	$C_{42}H_{62}O_{17}$	838.3987	$[M + H]^+$	839.40598	0.57	663.37644; 487.32988; 179.05516
63	48.91	Licorice-saponin B2	$C_{42}H_{64}O_{15}$	808.4244	$[M + H]^+$	809.43180	1.13	809.43180; 633.40026; 439.39439
64	49.28	Formononetin	$C_{16}H_{12}O_4$	268.0735	$[M - H]^-$	267.06628	0.54	267.06628; 252.04226; 195.04460
65	50.14	22-β-Acetoxylglyrrhaldehyde	$C_{44}H_{64}O_{17}$	864.4142	$[M + H]^+$	865.42163	−0.33	689.37723; 513.34358; 179.04966
					$[M - H]^-$	863.40707	2.76	481.33178; 353.07200; 193.03483
66	50.76	Glycyrrhizic acid	$C_{42}H_{62}O_{16}$	822.4037	$[M + H]^+$	823.41106	1.57	647.37952; 471.34743; 425.35761; 407.33922
67	50.79	Glycyrrhetinic acid	$C_{30}H_{46}O_4$	470.3396	$[M + H]^+$	471.34689	1.04	339.26538; 189.16722; 137.13835
68	54.41	Licorice-saponin K2	$C_{42}H_{62}O_{16}$	822.4037	$[M + H]^+$	823.41106	1.08	823.41106; 647.82600; 471.70200
					$[M - H]^-$	821.39651	1.37	821.39651; 646.55342
69	52.84	3′-Methoxyglabridin	$C_{21}H_{22}O_5$	354.1467	$[M - H]^-$	353.13945	0.27	353.13945; 338.15542; 147.04734
70	53.16	Licorice-saponin H2	$C_{42}H_{62}O_{16}$	822.4037	$[M + H]^+$	823.41106	1.08	823.41106; 647.37952; 471.34743
71	54.23	Licorice-saponin J2	$C_{42}H_{64}O_{16}$	824.4194	$[M + H]^+$	825.42671	1.53	825.42671; 649.39517; 455.40456
					$[M - H]^-$	823.41216	0.59	823.41216; 647.37952; 193.03483
72	53.65	Uralsaponin C	$C_{42}H_{64}O_{16}$	824.4194	$[M + H]^+$	825.42671	1.53	649.39517; 473.36309; 455.35252; 437.34196
73	53.96	Glycycoumarin	$C_{21}H_{20}O_6$	368.1259	$[M - H]^-$	367.11871	0.3	367.11817; 296.27800; 369.13811; 313.07121; 285.07630

[a] Ps: 1–30 from Paeoniae Radix Alba; 31–73 from Glycyrrhizae Radix et Rhizoma Praeparata.

of ingredients. A concrete illustration of the ingredients' characterization was performed as hereunder.

3.1 Characterization of the constituents in Paeoniae Radix Alba

Monoterpenes and several phenols were the primary active ingredients in Paeoniae Radix Alba with majority of them being monoterpenes. In this study, a tentative characterization of 30 compounds of Paeoniae Radix Alba in SGD was performed, followed by the identification of four of them. Peaks 1, 2, 3 and 6 in Fig. 1C can be attributed to gallic acid, albiflorin, paeoniflorin, and benzoyl paeoniflorin, respectively. Albiflorin was used as an illustration for demonstrating the fragmentation pathways of monoterpenes in Paeoniae Radix Alba. In the negative mode, the ion at *m*/*z* 479.15587 was inferred to be the adduct ion ($[M - H]^-$), followed by the calculation of the

formula as $C_{23}H_{28}O_{11}$. In the MS/MS spectrum, the key fragment ions found were at *m/z* 435.16551, 357.11856, and 121.02895, which suggested the loss of CO_2 (44 Da), $C_7H_5O_2$ (122 Da) and $C_{16}H_{22}O_9$ (358 Da) from the precursor ion, respectively. Glucogallin was selected as an illustration for demonstrating the fragmentation pathways of phenol. In respect to the negative mode, the ion at *m/z* 331.06707 was deducted to be the adduct ion ($[M - H]^-$) and the calculated formula was $C_{13}H_{16}O_{10}$. The important fragment ions found in the MS/MS spectrum were at *m/z* 313.05596, 211.02426, 169.01370 and 125.02387. The ion at *m/z* 313.05596 can be attributed to the loss of OH (17 Da) from the precursor ion, whereas the ion at *m/z* 211.02426 can be attributed to the loss of $C_4H_8O_4$ (120 Da) from the precursor ion. The ions at *m/z* 169.01370 and 125.02387 represented $C_7H_6O_5$ and $C_6H_6O_3$, respectively, and the characterization of other compounds in Paeoniae Radix Alba was performed based on fragmentation patterns and related literature.[10,12,13]

3.2 Characterization of constituents in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle

Triterpene saponins and flavonoids were the primary active constituents in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle.[14] In this research, tentative characterization of 43 ingredients of Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle in SGD was performed, followed by the precise identification of four among them. Peaks 4, 5, 7 and 8 in Fig. 1C represented liquiritin, ononin, isoliquiritigenin, and glycyrrhizic acid, respectively. Glycyrrhizic acid was used as a common triterpene saponins composition of Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle as an illustration for demonstrating the fragmentation pathways. In the positive mode, the ion at *m/z* 823.44106 was inferred to be the adduct ion ($[M + H]^+$), followed by the calculation of the formula as $C_{42}H_{62}O_{16}$. The key fragment ions found in the MS/MS spectrum were at *m/z* 647.37952, 471.34743, 425.35761 and 407.33922. The ion at *m/z* 647.37952 suggested the loss of $C_6H_8O_6$ (176 Da) from the precursor ion, that at *m/z* 471.34743 revealed the loss of $C_6H_8O_6$ (176 Da) from the *m/z* 647.37952, that at *m/z* 425.35761 suggested the loss of CHO_2 (46 Da) from the *m/z* 471.34743, and that at *m/z* 407.33922 revealed the loss of H_2O (18 Da) from the *m/z* 425.35761. Liquiritin was used as an example for demonstrating the fragmentation pathways of flavonoids in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle. In respect to the negative mode, the ion at *m/z* 417.11856 was confirmed to be the adduct ion ($[M - H]^-$), followed by the calculation of the formula as $C_{21}H_{22}O_9$. The key fragment ions found in the MS/MS spectrum were at *m/z* 255.06573, 153.05070, 135.00822 and 119.03231. The ion at *m/z* 255.06573 denoted the loss of $C_6H_{11}O_5$ (178 Da) from the precursor ion; that at *m/z* 153.05070 denoted the loss of C_7H_3O (102 Da) from the *m/z* 255.06573; that at *m/z* 135.00822 denoted the loss of C_8H_7O (120 Da) from the *m/z* 255.06573; and that at *m/z* 119.03231 denoted the loss of O (16 Da) from the *m/z* 135.00822. Characterization of the other ingredients in Glycyrrhizae Radix et Rhizoma was performed based on the fragmentation patterns and related literature.[14,16]

SGD is a classical formula of traditional Chinese medicine that is extensively used in the clinic due to its anti-inflammatory, immunoregulatory, analgesic, antidepression, hepatoprotective and neuroprotective effects.[12] Moreover, there is a wealth of study on the pharmacological effects of certain active components in the Paeoniae Radix Alba and Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle. This study revealed that monoterpenes and several phenols (in Paeoniae Radix Alba) and the triterpene saponins and flavonoids (in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle) constituted the key ingredients in SGD. Some of these chemical components have been reported to be the active ingredients in SGD.[8,10,13,17,18] For example, paeoniflorin was reported to have anti-inflammatory, hepatoprotective and neuroprotective effects.[19,20] Albiflorin was shown to be both anti-inflammatory and antioxidant.[9,21] Polyphenol was reported to play a role in antioxidant and antiviral activity. Pentagalloylglucose was shown to have anti-inflammatory, anti-allergic, antitumor, antiviral and antibacterial effects. Paeonol was reported to have anti-inflammatory, antitumor, anti-allergic, antioxidant activities, along with cardiovascular and neuroprotective effects.[22] Liquiritin had antidepressive and neuroprotective effects.[23,24] Liquiritigenin had been reported to exhibit anti-inflammatory effect.[25] Saponins from liquorice demonstrated anti-inflammatory, antiarrhythmia and hepatoprotective effects.[26,27] To better understand the major functional compounds and the mechanism of SGD, additional research is required. This study provides a good basis for identifying the prototype components and metabolites in SGD, which can better illustrate its medicinal value.

4 Conclusions

A rapid method was performed to systematically characterize 73 chemical constituents of SGD in total with the help of UHPLC-FT-ICR-MS. Experimental results reveal that phenols and monoterpenes (in Paeoniae Radix Alba), triterpene saponins and flavonoids (in Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle) are the primary components in SGD. Moreover, it provides more information about the compounds in SGD than the previous literature. Therefore, the results of this study can be used to evaluate the quality of SGD and provide a basis for subsequent *in vivo* studies of SGD. Furthermore, this work provides a method for rapid identification of other TCMs. However, additional studies are required to overcome the limitation of identifying only known compounds using this method.

Abbreviations

BPC	Base peak ion chromatograms
EIC	Extracted ion chromatograms
SGD	Shaoyao Gancao decoction
TCM	Traditional Chinese medicine

UHPLC-FT-ICR-MS/MS	Ultra high-performance liquid chromatography coupled with Fourier transform ion cyclotron resonance mass spectrometry

Conflicts of interest

The authors declare that there are no conflicts of interest.

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