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## Original Article

Rapid identification of chemical profile in Gandou decoction by UPLC-Q-TOF-MS<sup>E</sup> coupled with novel informatics UNIFI platformLi Xu <sup>a</sup>, Yi Liu <sup>a,c</sup>, Hongfei Wu <sup>b</sup>, Huan Wu <sup>b</sup>, Xiaochuang Liu <sup>b</sup>, An Zhou <sup>a,b,\*</sup><sup>a</sup> The Experimental Research Center, Anhui University of Chinese Medicine, Hefei 230038, China<sup>b</sup> Anhui Province Key Laboratory of Chinese Medicinal Formula, Hefei 230012, China<sup>c</sup> Waters Corporation (China), Shanghai 201206, China

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## ABSTRACT

Gandou decoction (GDD), a well-known traditional Chinese medicine (TCM) formula, has been widely used for decades to treat Wilson's disease (WD) in China due to its remarkable clinical effects. However, the chemical constituents of GDD still remain unclear because of their complexity. In this work, a reliable and sensitive strategy based on ultra-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS<sup>E</sup>) and UNIFI informatics platform was applied to investigate the chemical components in GDD. In total, 96 compounds including anthraquinones, alkaloids, protostane triterpenoids, flavonoids, triterpenoid saponins, tannins, curcuminoids, etc. were identified or tentatively characterized from GDD by comparing their retention time, accurate mass within 5 ppm error and MS<sup>E</sup> fragmentation patterns. Among them, eleven compounds were confirmed unambiguously with reference standards. Representative compounds in different chemical structure types were analyzed in fragmentation patterns and characteristic ions. Moreover, to better understand the chemical contribution of individual herbs to the whole decoction, the corresponding each herb in GDD was also detected. This study developed a rapid method for characterizing the chemical constituents in GDD, which could not only be used for chemical standardization and quality control, but also be helpful for further research of GDD in vivo.

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## 1. Introduction

Traditional Chinese medicines (TCM) have been extensively used for the prevention and treatment of complex and chronic diseases in China [1,2]. TCM formulae, combination of medicinal plants or animal materials, collectively exert therapeutic actions by complex interactions among multiple components from different herbal medicines. Based on TCM theories, these constituents in formulae could play a multi-target, synergistic and harmless therapeutic role [3]. As the components in TCM are rather complicated, it is difficult to separate and identify multiple chemical constituents. Therefore, developing a rapid and reliable method for elucidating the composition of TCM is necessary.

Wilson's disease (WD), also known as hepatolenticular

degeneration, is an autosomal recessive genetic disorder of copper metabolism caused by ATP7B gene mutation [4,5]. Excessive copper accumulation in patients suffering from WD leads to liver disease, neurological disorder, K-F rings, and osteoporosis [6]. Currently, there are several chelating agents such as D-penicillamine, dimercaptosuccinic acid, trientine, and tetrathiomolybdate for medical therapy [7]. Although Western conventional medications are highly effective, prevalent, and low-priced, a number of side effects have been observed with chelation therapy [8]. Gandou decoction (GDD), a classical TCM formula, has been used in clinics to treat WD for decades in China [9,10]. It is composed of six crude drugs, i.e., *Rheum palmatum* L. (Da-Huang), *Coptis chinensis* Franch. (Huang-Lian), *Curcuma longa* L. (Jiang-Huang), *Lysimachia christinae* Hance (Jin-Qian-Cao), *Alisma orientale* (Sam.) Juzep. (Ze-Xie) and *Panax notoginseng* (Burk.) F. H. Chen (San-Qi). The clinical studies have been proven that GDD can promote urinary copper excretion, ameliorate liver function and improve the patient's clinical symptoms [7,11]. Furthermore, GDD appears to be safe, effective, and well tolerated and has fewer adverse effects than Western conventional medications [12]. In our previous studies, we investigated

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the therapeutic effect and serum metabolic profiling of GDD in copper-laden rats. It was found that GDD could reduce the hepatic copper accumulation, and improve liver pathological characteristics by restoring the impaired lipid metabolism, amino metabolism and glucose metabolism [13]. However, due to multi-component systems of TCM, the chemical constituents of GDD still remain unclear. Therefore, a systematic chemical profiling research of GDD is in an urgent need.

In recent years, UPLC-Q-TOF-MS<sup>E</sup> (where E represents collision energy) has provided a powerful approach for the efficient separation and structural characterization of TCM with the advantage of its high resolution, sensitivity and accuracy [14]. Q-TOF-MS<sup>E</sup> capable of simultaneously acquiring accurate mass precursor ion in MS full scan and fragment ions in MS<sup>E</sup> high-energy scan increased the credibility of analysis results [15,16]. Additionally, UNIFI software from Waters Corporation is a versatile and automated data processing platform. The software incorporates scientific library into a streamlined workflow to integrate data acquisition, library searching, MS fragment matching and report generation, which alleviates the workload from massive MS data and realizes rapid analysis of chemical components [17]. This high throughput strategy was innovatively used for screening and identification of chemical components in herbal medicines [18,19] and TCM formulae [20]. In the present study, an integrative strategy based on UPLC-Q-TOF-MS<sup>E</sup> coupled with UNIFI informatics platform has been applied to reveal the chemical profile of GDD. The aim of this study is to develop an analytical method for elucidating the material basis of GDD and provide valuable information for the quality control and in vivo analysis.

## 2. Material and methods

### 2.1. Materials and reagents

*Rheum palmatum* L., *Coptis chinensis* Franch., *Curcuma longa* L., *Lysimachia christinae* Hance, *Alisma orientale* (Sam.) Juzep. and *Panax notoginseng* (Burk.) F. H. Chen were purchased from Beijing Tongrentang Co., Ltd. (Hefei, China) and authenticated by Doctor Rongchun Han (College of Pharmacy, Anhui University of Chinese Medicine, Hefei, China). All voucher specimens were deposited at the authors' laboratory. The reference standards, including berberine hydrochloride, phycion, emodin, alisol B 23-acetate, quercetin and notoginsenoside R<sub>1</sub>, were obtained from the National Institutes for Food and Drug Control (Beijing, China). Chrysophanol, rhein, aloe-emodin and kaempferide were obtained from Beina Chuanglian Biotechnology Research Institute (Beijing, China). Curcumin was isolated in our laboratory with a purity of more than 98% by HPLC, and its structure and molecular weight have been identified by using several spectral analyses and MS, respectively. Acetonitrile and methanol (LC-MS grade) were purchased from TEDIA (Fairfield, USA). Formic acid was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Ultrapure water was purified using a Milli-Q water purification system (Millipore, Billerica, MA, USA).

### 2.2. Standards and sample preparation

GDD consisted of six ingredients, including *Rheum palmatum* L. (20.0 g), *Coptis chinensis* Franch (20.0 g), *Curcuma longa* L. (20.0 g), *Lysimachia christinae* Hance (24.0 g), *Alisma orientale* (Sam.) Juzep. (24.0 g) and *Panax notoginseng* (Burk.) F. H. Chen (3.0 g). They were mixed together and immersed in 0.8 L distilled water (1:8, w/v) for 0.5 h. Afterwards, they were decocted twice by extracting and refluxing for 1 h each time. Finally, the two extractions were combined and concentrated to 1.0 g crude drug per milliliter, and

then the solution was freeze-dried and stored in a vacuum desiccator before use. The accurately weighed 1.0 g freeze-dried powder was dispersed in 30 mL methanol and ultrasonicated in a water bath for 30 min to prepare solutions. The individual preparation of six herbs was carried out according to the same procedures as that of GDD. An aliquot of 5  $\mu$ L filtrate was injected into the UPLC-Q-TOF-MS<sup>E</sup> system for analysis after filtered through 0.22  $\mu$ m filter membrane.

11 reference standards were dissolved in methanol. Before qualitative analysis, they were mixed together to make reasonable concentration and filtered through 0.22  $\mu$ m filter membrane.

### 2.3. Chromatography and mass spectrometry conditions

Chromatographic analysis was performed using a Waters Acuity<sup>TM</sup> UPLC system (Waters Corporation, Milford, USA). Chromatographic separation was carried out at 30 °C, using an Agilent Eclipse Plus C18 RRHD column (2.1 mm  $\times$  100 mm, 1.8  $\mu$ m) with mobile phases A (0.1% formic acid in water) and B (acetonitrile). The flow rate was set at 0.3 mL/min. The gradient profile was as follows: 0–1 min, 10%–10% B; 1–4 min, 10%–20% B; 4–10 min, 20%–30% B; 10–15 min, 30%–40% B; 15–18 min, 40%–50% B; 18–23 min, 50%–75% B; 23–25 min, 75%–85% B; 25–27 min, 85%–100% B.

Mass spectrometric detection was carried out on Waters Xevo G2 Q-TOF mass spectrometer (Waters Corporation, Milford, USA) equipped with an ESI source. The full scan data were acquired from 50 to 1200 Da, using a capillary voltage of 3.0 kV for positive ion mode and –2.5 kV for negative ion mode, sampling cone voltage of 40 V for positive ion mode and 50 V for negative ion mode, extraction cone voltage of 4.0 V, source temperature of 120 °C (ESI<sup>+</sup>) or 110 °C (ESI<sup>–</sup>), cone gas flow of 50 L/h, desolvation gas (N<sub>2</sub>) flow of 600 L/h and desolvation gas temperature of 350 °C. The collision voltage was set as 6.0 eV for low-energy scan and 20–80 eV for high-energy scan. Data were centroided and mass was corrected during acquisition using an external reference (LockSpray<sup>TM</sup>) consisting of a 200  $\mu$ g/mL solution of leucine-enkephalin infused at a flow rate of 10  $\mu$ L/min via a lockspray interface, generating a realtime reference ion of [M+H]<sup>+</sup> (*m/z* 556.2771) in positive ion mode and [M–H]<sup>–</sup> (*m/z* 554.2615) in negative ion mode to ensure accurate MS analysis. All data collected in centroid mode were obtained and used to calculate the accurate mass and composition of relative target ions with MassLynx<sup>TM</sup> V4.1 software (Waters).

### 2.4. Establishment of a chemical compounds library of GDD

The systematic information on chemical compounds isolated from the six individual herbs in GDD was collected and sorted out by retrieving databases such as China Journals of Full-text database (CNKI), Medline, PubMed, Web of Science and ChemSpider. A self-building library of chemical compounds was established by UNIFI software, including compound name, molecular formula, chemical structure, and accurate molecular mass. Among them, the information of 356 compounds is listed in Table S1.

### 2.5. Data analysis by UNIFI platform

All MS data analysis was processed on the platform of UNIFI software. Minimum peak area of 200 was set for 2D peak detection. The peaks intensity of high energy over 80 counts and the peak intensity of low energy over 200 counts were the selected parameters in 3D peak detection. A margin of error up to 5 ppm for identified compounds was allowed and the matching compounds would be generated predicted fragments from structure. We selected positive adducts including H<sup>+</sup>, Na<sup>+</sup> and negative adducts

containing  $\text{HCOO}^-$  and  $\text{H}^-$ . They were allowed cross adduct combinations.

### 3. Results and discussion

#### 3.1. Identification and characterization of chemical compounds

The high resolution MS data of GDD were quickly acquired by UPLC-Q-TOF-MS<sup>E</sup> method. The base peak intensity (BPI) chromatograms of GDD in positive and negative ion modes are depicted in Fig. 1. The UNIFI screening platform was utilized to process and analyze the MS data, and then automatically matched the fragment information. After further manual verification, a total of 96 compounds were identified or tentatively characterized in GDD, including 21 anthraquinones, 14 alkaloids, 17 protostane triterpenoids, 10 flavonoids, 8 triterpenoid saponins, 10 tannins, 4 curcuminoids and 12 others. The detailed MS information of these components is summarized in Table 1. Meanwhile, the chemical structures were confirmed based on accurate mass, MS<sup>E</sup> data and related literatures. The structures of main chemical constituents in GDD are shown in Fig. 2.

#### 3.2. Analysis of GDD by UPLC-Q-TOF-MS<sup>E</sup>

##### 3.2.1. Anthraquinones

21 anthraquinones were detected from GDD and were also the major bioactive constituents of *Rheum palmatum* L. In this study, 6 free anthraquinones, 13 anthraquinone glycosides and 2 anthrones were determined based on MS database-matching. Anthraquinones have a characteristic fragmentation behavior with successive or simultaneous losses of CO, OH, CH<sub>3</sub> and CO<sub>2</sub> [21,22]. Peaks 64, 65, 76, 83 and 86 were exactly identified as aloë emodin, rhein, emodin, chrysophanol and physcion by comparing retention time

and fragmentation patterns with reference standards. Rhein, the main anthraquinone in GDD, was used to characterize the fragmentation pathways (Fig. 3). Rhein showed quasi-molecular ion  $[\text{M}-\text{H}]^-$  at  $m/z$  283.0256 in negative ion mode, and yielded fragment ions at  $m/z$  239.0356 and 211.0403 by losses of CO<sub>2</sub> and CO, respectively. And then, the ion at  $m/z$  211.0403 could further lose one molecule of CO to generate ion at  $m/z$  183.0439. Aloë emodin and emodin were isomers with the same  $[\text{M}-\text{H}]^-$  ion at  $m/z$  269. In high energy MS<sup>E</sup> spectra, emodin revealed  $[\text{M}-\text{H}-\text{CO}]^-$  ion at  $m/z$  241.0507 and  $[\text{M}-\text{H}-\text{CO}-\text{O}]^-$  ion at  $m/z$  225.0559, while aloë emodin could be differentiated by the characteristic ion  $[\text{M}-\text{H}-\text{CHO}]^-$  at  $m/z$  240.0421. Physcion showed  $[\text{M}-\text{H}]^-$  ion at  $m/z$  283.0619, and the obvious fragments ions at  $m/z$  255.0315  $[\text{M}-\text{H}-\text{CO}]^-$  and 240.0359  $[\text{M}-\text{H}-\text{CO}-\text{CH}_3]^-$  were further obtained. Chrysophanol showed  $[\text{M}-\text{H}]^-$  ion at  $m/z$  253.0515, only one product ion at  $m/z$  225.0545  $[\text{M}-\text{H}-\text{CO}]^-$ .

For anthraquinone glycosides, aglycone ions were identified based on the MS fragmentation behaviors of free anthraquinones. Peak 47 exhibited  $[\text{M}-\text{H}]^-$  ion at  $m/z$  415.1045, which generated an  $[\text{M}-\text{H}-162\text{Da}]^-$  ion at  $m/z$  253.0515 by eliminating the glucose residue. The further loss of CO was in accordance with the characteristic ion at  $m/z$  225.0563 of chrysophanol. Thus, peak 47 was assigned as chrysophanol-1-*O*- $\beta$ -D-glc. Based on these fragmentation patterns, peaks 15, 17, 32, 35, 44, 45, 46, 47, 48, 51, 52, 53, and 60 were inferred as anthraquinones glycosides. In addition, anthrones are an important type of anthraquinone. Sennosides usually gave a significant ion at  $m/z$  386 which originated from C-10–C-10' cleavage. Peak 27 showed  $[\text{M}-\text{H}]^-$  ion at  $m/z$  861.1908, which first produced ions at  $m/z$  699.1358 and 655.1880 by sequential loss of terminal glucose residue and CO<sub>2</sub>, followed by the cleavage of C-10 and C-10' forming  $[\text{M}-\text{H}-\text{Glc}-\text{CO}_2-\text{C}_{15}\text{H}_9\text{O}_5]^-$  ion at  $m/z$  386.1002. Its structure was identified as sennoside A or B. These two isomers were not distinguished from each other only by their MS spectra. Similarly, peak 25 displayed the same fragmentation patterns as peak 27, so it was presumed as sennoside C or D.

##### 3.2.2. Alkaloids

A total of 14 alkaloids were identified in positive ion mode and came from *Coptis chinensis* Franch, including protoberberine alkaloids, apomorphine alkaloids, and tetrahydroprotoberberine alkaloids. As reported in the literature, the neutral losses like the methyl radical (CH<sub>3</sub>·), hydrogen radical (H·) and CO are the main fragment patterns of protoberberine alkaloids due to the successive cleavage of substituted methoxyl or methylenedioxy groups on the A- and D-rings [23]. Peak 39 was unequivocally identified as berberine by contrast with a reference standard. The MS spectrum and possible fragmentation pathways of berberine are depicted in Fig. 4. Taking berberine as an example, it produced fragment ions at  $m/z$  321.0983  $[\text{M}-\text{CH}_3]^+$ , 320.0918  $[\text{M}-\text{CH}_3-\text{H}]^+$ , 306.0763  $[\text{M}-2\text{CH}_3]^+$ , 292.0969  $[\text{M}-\text{CH}_3-\text{H}-\text{CO}]^+$  and 278.0813  $[\text{M}-2\text{CH}_3-\text{CO}]^+$ . Peak 40 showed  $[\text{M}]^+$  ion at  $m/z$  352.1538 and yielded characteristic ions at  $m/z$  337.1430  $[\text{M}-\text{CH}_3]^+$ , 336.0983  $[\text{M}-\text{CH}_3-\text{H}]^+$ , 322.1068  $[\text{M}-2\text{CH}_3]^+$  and 308.1275  $[\text{M}-\text{CH}_3-\text{H}-\text{CO}]^+$ , which indicated that it was presumed as palmatine. Likewise, the other peaks 31, 33, 34, 38 and 67 could be tentatively identified as coptisine, jatrorrhizine, epi-berberine, worenine and 8-oxoberberine, respectively.

Peak 10 gave  $[\text{M}]^+$  ion at  $m/z$  342.1701 with a molecular formula C<sub>20</sub>H<sub>24</sub>NO<sub>4</sub>. The predominant ions appeared at  $m/z$  297.1123  $[\text{M}-(\text{CH}_3)_2\text{NH}]^+$ , 282.0876  $[\text{M}-(\text{CH}_3)_2\text{NH}-\text{CH}_3]^+$ , 265.0861  $[\text{M}-(\text{CH}_3)_2\text{NH}-\text{CH}_3\text{OH}]^+$ , and 237.0908  $[\text{M}-(\text{CH}_3)_2\text{NH}-\text{CH}_3\text{OH}-\text{CO}]^+$ , which is consistent with the common structure of apomorphine alkaloids. Thus, peak 10 was considered as magnoflorine. Analogously, peaks 8 and 11 were deemed as isocorydine and isoboldine. Additionally, the tetrahydroprotoberberine alkaloids have retro-Diels-Alder (RDA) reaction, resulting in the cleavage of the

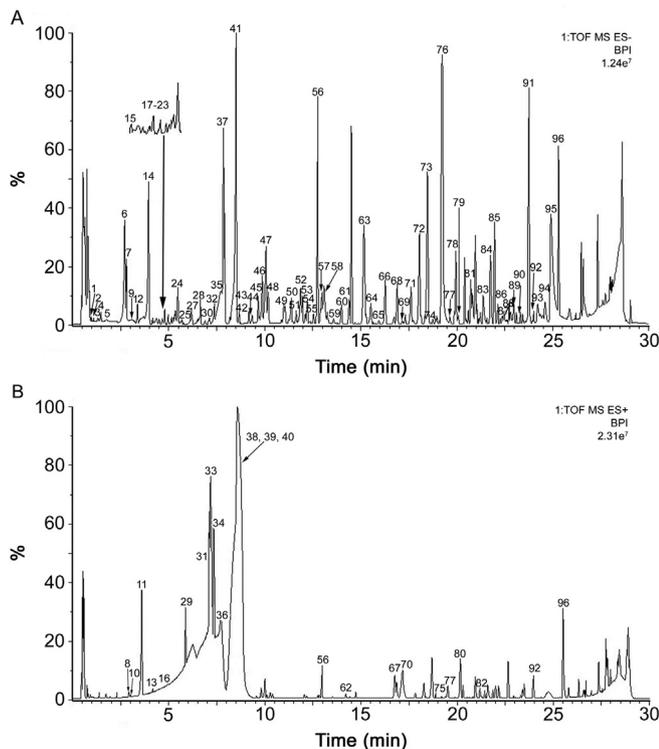


Fig. 1. The base peak intensity (BPI) chromatograms of GDD from UPLC-Q-TOF-MS<sup>E</sup> analysis. (A) Negative scan; (B) Positive scan.

**Table 1**  
Identification of chemical constituents of GDD by UPLC-Q-TOF-MS<sup>E</sup>.

No.	t <sub>R</sub> (min)	Identification	Neutral mass (Da)	Formular	Observed neutral mass (Da)	Experimental mass (m/z)	Error (ppm)	MS and MS <sup>E</sup> data (+ or -) (m/z)	Source
1	0.98	Gallic acid-O-glc	332.0743	C <sub>13</sub> H <sub>16</sub> O <sub>10</sub>	332.0743	331.0669	-0.5	331.0669[M-H] <sup>-</sup>	a
2	1.05	Gallic acid	170.0215	C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>	170.0214	169.0141	-0.8	169.0141[M-H-Glc] <sup>-</sup>	a
3	1.39	Progallin A	198.0528	C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>	198.0528	197.0455	-0.7	125.0244[M-H-CO <sub>2</sub> ] <sup>-</sup>	a
4	1.50	(+)-Catechin-5-O-β-D-glc	452.1319	C <sub>21</sub> H <sub>24</sub> O <sub>11</sub>	452.1318	451.1245	-0.7	197.0455[M-H] <sup>-</sup>	a
5	1.71	Procyanidin B	578.1424	C <sub>30</sub> H <sub>26</sub> O <sub>12</sub>	578.1432	577.1359	1.4	124.0455[M-H-2CH <sub>2</sub> -COOH] <sup>-</sup>	a
6	2.71	Chlorogenic acid	354.0951	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	354.0962	353.0889	3.1	451.1245[M-H] <sup>-</sup>	b,d
7	2.82	(-)-Epicatechin	290.0790	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	290.0797	289.0724	2.3	289.0717[M-H-Glc] <sup>-</sup>	a
8	3.16	Isocorydine	341.1627	C <sub>20</sub> H <sub>23</sub> NO <sub>4</sub>	341.1628	342.1707	0.1	425.0873[M-H-C <sub>7</sub> H <sub>4</sub> O <sub>4</sub> ] <sup>-</sup>	b
9	3.19	Procyanidin B-O-gallate	730.1534	C <sub>37</sub> H <sub>30</sub> O <sub>16</sub>	730.1544	729.1471	1.3	407.0763[M-H-C <sub>7</sub> H <sub>4</sub> O <sub>4</sub> -H <sub>2</sub> O] <sup>-</sup>	a
10	3.20	Magnoflorine	342.1705	C <sub>20</sub> H <sub>24</sub> NO <sub>4</sub>	342.1730	342.1701	-1.2	289.0712[M-H-catechin] <sup>-</sup>	b
11	3.42	Isoboldine	327.1471	C <sub>19</sub> H <sub>21</sub> NO <sub>4</sub>	327.1479	328.1552	2.7	577.1359[M-H-gallate] <sup>-</sup>	b
12	3.79	Isovitexin	432.1056	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	432.1065	477.1047	1.8	342.1701[M] <sup>+</sup>	d
13	4.75	Tetradehydroscoulerine	322.1079	C <sub>19</sub> H <sub>16</sub> NO <sub>4</sub>	322.1055	322.1052	-6.6	297.1123[M-(CH <sub>3</sub> ) <sub>2</sub> NH] <sup>+</sup>	b
14	4.79	Procyanidin B-5,3,3'-di-O-gallate	882.1643	C <sub>44</sub> H <sub>34</sub> O <sub>20</sub>	882.1685	881.1612	4.8	282.0876[M-(CH <sub>3</sub> ) <sub>2</sub> NH-CH <sub>3</sub> ] <sup>+</sup>	a
15	4.86	Emodin-8-O-β-D-glc	432.1056	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	432.1065	431.0992	1.9	265.0861[M-(CH <sub>3</sub> ) <sub>2</sub> NH-CH <sub>3</sub> OH] <sup>+</sup>	a
16	4.88	Rutin	610.1534	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	610.1555	609.1483	3.6	237.0908[M-(CH <sub>3</sub> ) <sub>2</sub> NH-CH <sub>3</sub> OH-CO] <sup>+</sup>	a,d
17	5.09	Rhein-1-O-β-D-glc	446.0849	C <sub>21</sub> H <sub>18</sub> O <sub>11</sub>	446.0860	445.0787	2.4	328.1552[M+H] <sup>+</sup>	a
18	5.11	Isolindleyin or lindleyin	478.1475	C <sub>23</sub> H <sub>26</sub> O <sub>11</sub>	478.1483	477.1410	1.7	297.1123[M+H-CH <sub>3</sub> O] <sup>+</sup>	d
19	5.14	(+)-Catechin-3-O-gallate	442.0900	C <sub>22</sub> H <sub>18</sub> O <sub>10</sub>	442.0910	441.0838	2.4	477.1047[M+HCOO] <sup>-</sup>	a
								431.0982[M-H] <sup>-</sup>	
								311.0567[M-H-CO <sub>2</sub> -C <sub>3</sub> H <sub>8</sub> O <sub>2</sub> ] <sup>-</sup>	
								322.1052[M] <sup>+</sup>	
								307.0839[M-CH <sub>3</sub> ] <sup>+</sup>	
								279.0511[M-CH <sub>3</sub> -CO] <sup>+</sup>	
								881.1612[M-H] <sup>-</sup>	
								729.1471[M-H-gallate] <sup>-</sup>	
								711.0735[M-H-gallate-H <sub>2</sub> O] <sup>-</sup>	
								559.1341[M-H-2gallate-H <sub>2</sub> O] <sup>-</sup>	
								431.0992[M-H] <sup>-</sup>	
								269.0461[M-H-Glc] <sup>-</sup>	
								241.0493[M-H-Glc-CO] <sup>-</sup>	
								225.0554[M-H-Glc-CO-O] <sup>-</sup>	
								609.1483[M-H] <sup>-</sup>	
								463.0815[M-H-Rha] <sup>-</sup>	
								301.0487[M-H-Rha-Glc] <sup>-</sup>	
								445.0787[M-H] <sup>-</sup>	
								283.0256[M-H-Glc] <sup>-</sup>	
								239.0356[M-H-Glc-CO <sub>2</sub> ] <sup>-</sup>	
								211.0403[M-H-Glc-CO <sub>2</sub> -CO] <sup>-</sup>	
								477.1410[M-H] <sup>-</sup>	
								313.0552[M-H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ] <sup>-</sup>	
								169.0145[M-H-C <sub>16</sub> H <sub>20</sub> O <sub>6</sub> ] <sup>-</sup>	
								441.0838[M-H] <sup>-</sup>	
								289.0722[M-H-C <sub>7</sub> H <sub>4</sub> O <sub>4</sub> ] <sup>-</sup>	
								165.1245[M-H-C <sub>14</sub> H <sub>12</sub> O <sub>6</sub> ] <sup>-</sup>	

20	5.20	Ferulic acid- <i>O</i> - $\beta$ -D-glc	356.1107	C <sub>16</sub> H <sub>20</sub> O <sub>9</sub>	356.1113	355.1040	1.5	355.1040[M-H] <sup>-</sup>	d
21	5.25	Isoquercitrin	464.0955	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	464.0972	463.0899	3.7	193.1033[M-H-Glc] <sup>-</sup> 463.0899[M-H] <sup>-</sup>	d
22	5.45	Isolindleyin or lindleyin	478.1475	C <sub>23</sub> H <sub>26</sub> O <sub>11</sub>	478.1482	477.1408	1.6	301.0038[M-H-C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ] <sup>-</sup> 477.1408[M-H] <sup>-</sup>	a
23	5.61	Kaempferol-3- <i>O</i> -rutinoside	594.1585	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	594.1599	593.1521	1.6	313.0551[M-H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ] <sup>-</sup> 169.0144[M-H-C <sub>16</sub> H <sub>20</sub> O <sub>6</sub> ] <sup>-</sup> 593.1521[M-H] <sup>-</sup>	d
24	5.65	Resveratrol-4'- <i>O</i> - $\beta$ -D-(2''- <i>O</i> -galloyl)-glc	542.1424	C <sub>27</sub> H <sub>26</sub> O <sub>12</sub>	542.1431	541.1333	1.2	447.1591[M-H-Rha] <sup>-</sup> 285.0832[M-H-Rha-Glc] <sup>-</sup> 541.1333[M-H] <sup>-</sup>	a
25	5.70	Senoside C or D	848.2164	C <sub>42</sub> H <sub>40</sub> O <sub>19</sub>	848.2177	847.2104	1.5	313.0555[M-H-Res-H <sub>2</sub> O] <sup>-</sup> 847.2104[M-H] <sup>-</sup>	a
26	5.89	Tetrahydropalmatine	355.1784	C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>	355.1792	356.1865	2.4	685.1557[M-H-Glc] <sup>-</sup> 356.1865[M+H] <sup>+</sup>	b
27	6.24	Senoside A or B	862.1956	C <sub>42</sub> H <sub>38</sub> O <sub>20</sub>	862.1981	861.1908	2.8	192.1019[M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ] <sup>+</sup> 861.1908[M-H] <sup>-</sup>	a
28	6.36	Homoorientin	448.1006	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	448.1000	447.0927	-1.2	699.1358[M-H-Glc] <sup>-</sup> 655.1880[M-H-Glc-CO <sub>2</sub> ] <sup>-</sup> 386.1002[M-H-Glc-CO <sub>2</sub> -C <sub>15</sub> H <sub>9</sub> O <sub>5</sub> ] <sup>-</sup>	d
29	6.52	Thalifendine	322.1079	C <sub>19</sub> H <sub>16</sub> NO <sub>4</sub>	322.1069	322.1082	-0.4	447.0927[M-H] <sup>-</sup> 322.1082[M] <sup>+</sup>	b
30	6.94	Hesperetin-5- <i>O</i> -glc	464.1319	C <sub>22</sub> H <sub>24</sub> O <sub>11</sub>	464.1313	463.1240	1.5	279.0889[M-CH <sub>3</sub> -CO] <sup>+</sup> 463.1240[M-H] <sup>-</sup>	d
31	7.01	Coptisine	320.0923	C <sub>19</sub> H <sub>14</sub> NO <sub>4</sub>	320.0921	320.0921	0.6	301.1131[M-H-Glc] <sup>-</sup> 320.0921[M] <sup>+</sup>	b
32	7.08	Rhein-8- <i>O</i> - $\beta$ -D-(6''- <i>O</i> -acetyl)-glc	488.0955	C <sub>23</sub> H <sub>20</sub> O <sub>12</sub>	488.0949	487.0876	1.5	292.0970[M-CO] <sup>+</sup> 487.0876[M-H] <sup>-</sup>	a
33	7.15	Jatrorrhizine	338.1392	C <sub>20</sub> H <sub>20</sub> NO <sub>4</sub>	338.1384	338.1384	2.4	283.0246[M-H-acetyl-Glc] <sup>-</sup> 239.0347[M-H-acetyl-Glc-CO <sub>2</sub> ] <sup>-</sup> 338.1384[M] <sup>+</sup>	b
34	7.35	Epiberberine	336.1236	C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub>	336.1250	336.1250	-4.2	322.0997[M-CH <sub>3</sub> -H] <sup>+</sup> 308.1309[M-2CH <sub>3</sub> ] <sup>+</sup> 336.1250[M] <sup>+</sup>	b
35	7.47	Aloe emodin-8- <i>O</i> - $\beta$ -D-glc	432.1056	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	432.1068	431.0992	0.8	320.0935[M-CH <sub>3</sub> -H] <sup>+</sup> 292.1314[M-CH <sub>3</sub> -H-CO] <sup>+</sup> 431.0992[M-H] <sup>-</sup>	a
36	7.48	Tetrahydroberberine	339.1471	C <sub>20</sub> H <sub>21</sub> NO <sub>4</sub>	339.1466	340.1544	-1.4	269.0385[M-H-Glc] <sup>-</sup> 225.0562[M-H-Glc-CO <sub>2</sub> ] <sup>-</sup> 340.1544[M+H] <sup>+</sup>	b
37 <sup>S</sup>	7.72	Notoginsenoside R <sub>1</sub>	932.5345	C <sub>47</sub> H <sub>80</sub> O <sub>18</sub>	932.5372	977.5354	2.8	176.0705[M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ] <sup>+</sup> 977.5354[M+HCOO] <sup>-</sup> 931.5297[M-H] <sup>-</sup>	f
38	8.09	Worenine	334.1079	C <sub>20</sub> H <sub>16</sub> NO <sub>4</sub>	334.1080	334.1080	-0.3	799.4856[M-H-Xyl] <sup>-</sup> 637.4314[M-H-Xyl-Glc] <sup>-</sup> 475.3782[M-H-Xyl-2Glc] <sup>-</sup> 334.1080[M] <sup>+</sup>	b
39 <sup>S</sup>	8.46	Berberine	336.1236	C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub>	336.1230	336.1231	1.8	320.1093[M-CH <sub>2</sub> ] <sup>+</sup> 318.1092[M-CH <sub>2</sub> -2H] <sup>+</sup> 292.0969[M-CH <sub>2</sub> -CO] <sup>+</sup> 336.1231[M] <sup>+</sup>	b
40	8.51	Palmatine	352.1549	C <sub>21</sub> H <sub>22</sub> NO <sub>4</sub>	352.1543	352.1538	-1.7	321.0983[M-CH <sub>3</sub> ] <sup>+</sup> 320.0918[M-CH <sub>3</sub> -H] <sup>+</sup> 306.0763[M-2CH <sub>3</sub> ] <sup>+</sup> 292.0969[M-CH <sub>3</sub> -H-CO] <sup>+</sup> 278.0813[M-2CH <sub>3</sub> -CO] <sup>+</sup> 352.1538[M] <sup>+</sup>	b
								337.1430[M-CH <sub>3</sub> ] <sup>+</sup> 336.0983[M-CH <sub>3</sub> -H] <sup>+</sup> 322.1068 [M-2CH <sub>3</sub> ] <sup>+</sup> 308.1275 [M-CH <sub>3</sub> -H-CO] <sup>+</sup>	

Table 1 (continued)

No.	t <sub>R</sub> (min)	Identification	Neutral mass (Da)	Formular	Observed neutral mass (Da)	Experimental mass (m/z)	Error (ppm)	MS and MS <sup>E</sup> data (+ or -) (m/z)	Source
41	8.53	Ginsenoside Rg <sub>1</sub>	800.4922	C <sub>42</sub> H <sub>72</sub> O <sub>14</sub>	800.4939	845.4921	2.0	845.4921[M+HCOO] <sup>-</sup> 799.4863[M-H] <sup>-</sup> 637.4321[M-H-Glc] <sup>-</sup> 475.3784[M-H-2Glc] <sup>-</sup>	f
42	9.23	Kaempferol	286.0477	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	286.0479	285.0406	0.7	285.0406[M-H] <sup>-</sup> 229.0451[M-H-C <sub>2</sub> O <sub>2</sub> ] <sup>-</sup> 169.0141[M-H-C <sub>3</sub> O <sub>5</sub> ] <sup>-</sup>	a,d
43 <sup>g</sup>	9.46	Quercetin	302.0427	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	302.0432	301.0360	2.0	301.0360[M-H] <sup>-</sup> 151.0451[M-H-C <sub>8</sub> H <sub>6</sub> O <sub>3</sub> ] <sup>-</sup> 121.0431[M-H-C <sub>8</sub> H <sub>6</sub> O <sub>3</sub> -CH <sub>2</sub> O] <sup>-</sup> 107.0162[M-H-C <sub>8</sub> H <sub>6</sub> O <sub>3</sub> -CH <sub>2</sub> O-CH <sub>2</sub> ] <sup>-</sup>	d
44	9.61	Aloin	418.1264	C <sub>21</sub> H <sub>22</sub> O <sub>9</sub>	418.1267	417.1195	0.8	417.1195[M-H] <sup>-</sup> 255.0666[M-H-Glc] <sup>-</sup> 227.0718[M-H-Glc-CO] <sup>-</sup>	a
45	9.76	Aloe emodin-1-O-β-D-glc	432.1056	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	432.1068	431.0995	2.7	431.0995[M-H] <sup>-</sup> 269.0452[M-H-Glc] <sup>-</sup> 225.0562[M-H-Glc-CO <sub>2</sub> ] <sup>-</sup>	a
46	9.91	Aloe emodin-ω-O-β-D-glc	432.1056	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	432.1068	431.0995	2.7	431.0995[M-H] <sup>-</sup> 269.0463[M-H-Glc] <sup>-</sup> 225.0560[M-H-Glc-CO <sub>2</sub> ] <sup>-</sup>	a
47	10.09	Chrysophanol-1-O-β-D-glc	416.1107	C <sub>21</sub> H <sub>20</sub> O <sub>9</sub>	416.1118	415.1045	2.6	415.1045[M-H] <sup>-</sup> 253.0515[M-H-Glc] <sup>-</sup> 225.0563[M-H-Glc-CO] <sup>-</sup>	a
48	10.91	Aloe emodin-8-(6"-O-malonyl)-glc	518.1060	C <sub>24</sub> H <sub>22</sub> O <sub>13</sub>	518.1076	517.1003	3.1	517.1003[M-H] <sup>-</sup> 473.1097[M-H-CO <sub>2</sub> ] <sup>-</sup> 269.0463[M-H-malonyl-Glc] <sup>-</sup>	a
49	10.95	Calebin-A	384.1209	C <sub>21</sub> H <sub>20</sub> O <sub>7</sub>	384.1211	429.1193	0.5	429.1193[M+HCOO] <sup>-</sup>	c
50	11.16	Naringenin	272.0685	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	272.0689	271.0616	1.5	271.0616[M-H] <sup>-</sup> 151.0665[M-H-C <sub>8</sub> H <sub>8</sub> O] <sup>-</sup>	d
51	11.25	Aloe emodin-8-O-β-D-(6"-O-acetyl)-glc	474.1162	C <sub>23</sub> H <sub>22</sub> O <sub>11</sub>	474.1169	473.1096	1.5	473.1096[M-H] <sup>-</sup> 269.0451[M-H-acetyl-Glc] <sup>-</sup> 240.0410[M-H-acetyl-Glc-CHO] <sup>-</sup>	a
52	11.77	Physcion-8-O-β-D-glc	446.1213	C <sub>22</sub> H <sub>22</sub> O <sub>10</sub>	446.1219	445.1146	1.3	445.1146[M-H] <sup>-</sup> 283.0617[M-H-Glc] <sup>-</sup> 240.0433[M-H-Glc-CO-CH <sub>3</sub> ] <sup>-</sup>	a
53	11.85	Chrysophanol-8-O-β-D-(6"-O-acetyl)-glc	458.1213	C <sub>23</sub> H <sub>22</sub> O <sub>10</sub>	458.1221	457.1148	1.8	457.1148[M-H] <sup>-</sup> 253.0511[M-H-acetyl-Glc] <sup>-</sup> 225.0561[M-H-acetyl-Glc-CO] <sup>-</sup>	a
54	12.18	Notoginsenoside R <sub>2</sub>	770.4816	C <sub>41</sub> H <sub>70</sub> O <sub>13</sub>	770.4843	815.4825	3.3	815.4825[M+HCOO] <sup>-</sup> 769.4768[M-H] <sup>-</sup> 637.4335[M-H-Xyl] <sup>-</sup> 475.3786[M-H-Xyl-Glc] <sup>-</sup>	f
55	12.53	Ginsenoside Rb <sub>1</sub>	1108.6029	C <sub>54</sub> H <sub>92</sub> O <sub>23</sub>	1108.6105	1107.5967	6.6	1107.5967[M-H] <sup>-</sup> 945.5469[M-H-Glc] <sup>-</sup> 783.4917[M-H-2Glc] <sup>-</sup> 621.4361[M-H-3Glc] <sup>-</sup> 459.3824[M-H-4Glc] <sup>-</sup>	f
56	12.69	Cyclocurcumin	368.1260	C <sub>21</sub> H <sub>20</sub> O <sub>6</sub>	368.1258	367.1186	-0.4	367.1186[M-H] <sup>-</sup> 175.0401[M-H-C <sub>11</sub> H <sub>12</sub> O <sub>3</sub> ] <sup>-</sup>	c
57	12.74	Ginsenoside Rg <sub>2</sub>	784.4973	C <sub>42</sub> H <sub>72</sub> O <sub>13</sub>	784.4999	829.4982	3.2	829.4982[M+HCOO] <sup>-</sup> 783.4922[M-H] <sup>-</sup> 637.4326[M-H-Rha] <sup>-</sup> 475.3789[M-H-Rha-Glc] <sup>-</sup>	f

58	12.94	Ginsenoside Rh <sub>1</sub>	638.4394	C <sub>36</sub> H <sub>62</sub> O <sub>9</sub>	638.4414	683.4382	2.9	683.4382[M+HCOO] <sup>-</sup> 637.4321[M-H] <sup>-</sup>	f
59	13.18	6-Methyl-rhein	298.0477	C <sub>16</sub> H <sub>10</sub> O <sub>6</sub>	298.0481	297.0408	1.1	475.3784[M-H-Glc] <sup>-</sup> 297.0408[M-H] <sup>-</sup> 283.0251[M-H-CH <sub>2</sub> ] <sup>-</sup> 239.0358[M-H-CH <sub>2</sub> -CO <sub>2</sub> ] <sup>-</sup>	a
60	13.21	Physcion-8-O-β-D-(6"-O-acetyl)-glc	488.1319	C <sub>24</sub> H <sub>24</sub> O <sub>11</sub>	488.1323	487.1250	0.8	487.1250[M-H] <sup>-</sup> 283.0615[M-H-acetyl-Glc] <sup>-</sup> 255.0317[M-H-acetyl-Glc-CO] <sup>-</sup> 240.0432[M-H-acetyl-Glc-CO-CH <sub>3</sub> ] <sup>-</sup>	a
61	13.51	Orientalol A	254.1882	C <sub>15</sub> H <sub>26</sub> O <sub>3</sub>	254.1907	299.1889	8.3	299.1889[M+HCOO] <sup>-</sup> 253.1905[M-H] <sup>-</sup>	c
62	14.13	16-Oxo-alisol A	504.3451	C <sub>30</sub> H <sub>48</sub> O <sub>6</sub>	504.3474	505.3223	4.3	505.3223[M+H] <sup>+</sup> 487.3327[M+H-H <sub>2</sub> O] <sup>+</sup> 469.3285[M+H-2H <sub>2</sub> O] <sup>+</sup> 451.3318[M+H-3H <sub>2</sub> O] <sup>+</sup> 415.2824[M+H-C <sub>4</sub> H <sub>10</sub> O <sub>2</sub> ] <sup>+</sup> 397.2783[M+H-C <sub>4</sub> H <sub>10</sub> O <sub>2</sub> -H <sub>2</sub> O] <sup>+</sup>	e
63	14.41	Ginsenoside Rd	946.5501	C <sub>48</sub> H <sub>82</sub> O <sub>18</sub>	946.5581	991.5563	8.0	991.5563[M+HCOO] <sup>-</sup> 945.5563[M-H] <sup>-</sup> 783.4939[M-H-Glc] <sup>-</sup> 621.4398[M-H-2Glc] <sup>-</sup> 459.3851[M-H-3Glc] <sup>-</sup>	f
64 <sup>s</sup>	14.52	Aloe emodin	270.0528	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	270.0536	269.0462	3.0	269.0462[M-H] <sup>-</sup> 240.0421[M-H-CHO] <sup>-</sup> 211.0410[M-H-2CHO] <sup>-</sup>	a
65 <sup>s</sup>	15.30	Rhein	284.0321	C <sub>15</sub> H <sub>8</sub> O <sub>6</sub>	284.0332	283.0256	4.0	283.0256[M-H] <sup>-</sup> 239.0356[M-H-CO <sub>2</sub> ] <sup>-</sup> 211.0403[M-H-CO <sub>2</sub> -CO] <sup>-</sup> 183.0439[M-H-CO <sub>2</sub> -CO-CO] <sup>-</sup>	a
66	16.01	Notoginsenoside R <sub>3</sub>	962.5450	C <sub>48</sub> H <sub>82</sub> O <sub>19</sub>	962.5491	961.5418	4.2	961.5418[M-H] <sup>-</sup> 799.4889[M-H-Glc] <sup>-</sup> 637.4360[M-H-2Glc] <sup>-</sup> 475.3817[M-H-3Glc] <sup>-</sup>	f
67	16.70	8-Oxoberberine	351.1107	C <sub>20</sub> H <sub>17</sub> NO <sub>5</sub>	351.1108	352.1181	0.4	352.1181[M+H] <sup>+</sup> 337.0948[M+H-CH <sub>3</sub> ] <sup>+</sup> 294.0764[M+H-2CH <sub>3</sub> -CO] <sup>+</sup>	b
68	16.72	Alisol I	454.3447	C <sub>30</sub> H <sub>46</sub> O <sub>3</sub>	454.3460	455.3526	2.6	455.3526[M+H] <sup>+</sup> 383.2943[M+H-C <sub>4</sub> H <sub>8</sub> O] <sup>+</sup>	e
69	17.05	Alisol C	486.3345	C <sub>30</sub> H <sub>46</sub> O <sub>5</sub>	486.3368	487.3418	4.2	487.3418[M+H] <sup>+</sup> 469.3265[M+H-H <sub>2</sub> O] <sup>+</sup> 451.3215[M+H-2H <sub>2</sub> O] <sup>+</sup> 415.2841[M+H-C <sub>4</sub> H <sub>8</sub> O] <sup>+</sup> 397.2746[M+H-C <sub>4</sub> H <sub>10</sub> O <sub>2</sub> ] <sup>+</sup>	e
70	17.24	Ar-turmerone	216.1514	C <sub>15</sub> H <sub>20</sub> O	216.1516	217.1588	0.7	217.1588[M+H] <sup>+</sup> 120.0935[M+H-C <sub>6</sub> H <sub>9</sub> O] <sup>+</sup> 92.06212[M+H-C <sub>8</sub> H <sub>13</sub> O] <sup>+</sup>	c
71 <sup>s</sup>	17.34	Kaempferide	300.0634	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	300.0628	299.0553	-2.1	299.0553[M-H] <sup>-</sup> 284.0489[M-H-CH <sub>3</sub> ] <sup>-</sup>	d
72	17.46	Bisdemethoxycurcumin	308.1049	C <sub>19</sub> H <sub>16</sub> O <sub>4</sub>	308.1058	307.0984	2.9	307.0984[M-H] <sup>-</sup> 187.0406[M-H-C <sub>8</sub> H <sub>8</sub> O] <sup>-</sup>	c
73	17.93	Demethoxycurcumin	338.1154	C <sub>20</sub> H <sub>18</sub> O <sub>5</sub>	338.1161	337.1088	2.0	337.1088[M-H] <sup>-</sup> 217.0508[M-H-C <sub>8</sub> H <sub>8</sub> O] <sup>-</sup>	c
74 <sup>s</sup>	18.38	Curcumin	368.1260	C <sub>21</sub> H <sub>20</sub> O <sub>6</sub>	368.1260	367.1192	-0.1	367.1192[M-H] <sup>-</sup> 217.0524[M-H-C <sub>9</sub> H <sub>10</sub> O <sub>2</sub> ] <sup>-</sup>	c
75	18.94	Alisol M 23-acetate	488.3502	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3497	489.3480	-0.8	489.3480[M+H] <sup>+</sup> 471.2696[M+H-H <sub>2</sub> O] <sup>+</sup>	e

(continued on next page)

Table 1 (continued)

No.	t <sub>R</sub> (min)	Identification	Neutral mass (Da)	Formular	Observed neutral mass (Da)	Experimental mass (m/z)	Error (ppm)	MS and MS <sup>E</sup> data (+ or -) (m/z)	Source
76 <sup>§</sup>	19.25	Emodin	270.0528	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	270.0533	269.0460	1.7	269.0460[M-H] <sup>-</sup> 241.0507[M-H-CO] <sup>-</sup> 225.0559[M-H-CO-O] <sup>-</sup>	a
77	19.54	Bisabolone oxide A	236.1776	C <sub>15</sub> H <sub>24</sub> O <sub>2</sub>	236.1776	237.1850	0.5	237.1850[M+H] <sup>+</sup>	c
78	19.65	Alisol C 23-acetate	528.3451	C <sub>32</sub> H <sub>48</sub> O <sub>6</sub>	528.3453	529.3535	1.0	529.3535[M+H] <sup>+</sup> 511.3415[M+H-H <sub>2</sub> O] <sup>+</sup> 469.3456[M+H-HAc] <sup>+</sup>	e
79	19.71	Alisol F	488.3502	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3497	511.3391	-1.4	511.3391[M+Na] <sup>+</sup> 471.2696[M+H-H <sub>2</sub> O] <sup>+</sup> 453.2687[M+H-2H <sub>2</sub> O] <sup>+</sup> 399.3129[M+H-C <sub>4</sub> H <sub>10</sub> O <sub>2</sub> ] <sup>+</sup> 381.3091[M+H-C <sub>4</sub> H <sub>10</sub> O <sub>2</sub> -H <sub>2</sub> O] <sup>+</sup>	e
80	20.04	Alisol L 23-acetate	510.3345	C <sub>32</sub> H <sub>46</sub> O <sub>5</sub>	510.3350	511.3418	1.0	511.3418[M+H] <sup>+</sup> 451.3201[M+H-HAc] <sup>+</sup> 397.2739[M+H-C <sub>6</sub> H <sub>10</sub> O <sub>2</sub> ] <sup>+</sup>	e
81	20.59	Procyanidin	594.1373	C <sub>30</sub> H <sub>26</sub> O <sub>13</sub>	594.1382	593.1309	0.8	593.1309[M-H] <sup>-</sup>	a
82	21.39	Neolisol A	488.3502	C <sub>30</sub> H <sub>48</sub> O <sub>5</sub>	488.3505	489.3572	2.5	489.3572[M+H] <sup>+</sup> 471.3571[M+H-H <sub>2</sub> O] <sup>+</sup>	e
83 <sup>§</sup>	21.71	Chrysophanol	254.0579	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	254.0579	253.0515	3.3	253.0515[M-H] <sup>-</sup> 225.0545[M-H-CO] <sup>-</sup>	a
84	21.72	16,23-Oxido alisol B	470.3396	C <sub>30</sub> H <sub>46</sub> O <sub>4</sub>	470.3395	493.3287	-0.2	493.3287[M+Na] <sup>+</sup> 453.3362[M+H-H <sub>2</sub> O] <sup>+</sup> 339.2686[M+H-C <sub>6</sub> H <sub>10</sub> O <sub>2</sub> -H <sub>2</sub> O] <sup>+</sup>	e
85	22.56	Gingerdione	292.1675	C <sub>17</sub> H <sub>24</sub> O <sub>4</sub>	292.1679	337.1661	1.4	337.1661[M+HCOO] <sup>-</sup> 291.1629[M-H] <sup>-</sup>	c
86 <sup>§</sup>	22.80	Physcion	284.0685	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	284.0692	283.0619	2.5	283.0619[M-H] <sup>-</sup> 255.0315[M-H-CO] <sup>-</sup> 240.0359[M-H-CO-CH <sub>3</sub> ] <sup>-</sup>	a
87	22.90	Alisol A 23-acetate	532.3764	C <sub>32</sub> H <sub>52</sub> O <sub>6</sub>	532.3767	555.3749	0.6	555.3749[M+Na] <sup>+</sup> 515.3516[M+H-H <sub>2</sub> O] <sup>+</sup> 383.2797[M+H-C <sub>6</sub> H <sub>12</sub> O <sub>3</sub> -H <sub>2</sub> O] <sup>+</sup>	e
88	23.05	11-Deoxy-alisol C	470.3396	C <sub>30</sub> H <sub>46</sub> O <sub>4</sub>	470.3398	471.3380	0.3	471.3380[M+H] <sup>+</sup> 453.3515[M+H-H <sub>2</sub> O] <sup>+</sup> 399.2874[M+H-C <sub>4</sub> H <sub>8</sub> O] <sup>+</sup>	e
89	23.32	Stearic acid	284.2715	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284.2717	283.2645	0.7	283.2645[M-H] <sup>-</sup>	d
90	23.54	Alisol A	490.3658	C <sub>30</sub> H <sub>50</sub> O <sub>5</sub>	490.3678	491.3661	3.8	491.3661[M+H] <sup>+</sup> 473.3515[M+H-H <sub>2</sub> O] <sup>+</sup> 455.3621[M+H-2H <sub>2</sub> O] <sup>+</sup> 437.3415[M+H-3H <sub>2</sub> O] <sup>+</sup> 383.2973[M+H-H <sub>2</sub> O-C <sub>4</sub> H <sub>10</sub> O <sub>2</sub> ] <sup>+</sup>	e
91	23.79	Alisol B	472.3553	C <sub>30</sub> H <sub>48</sub> O <sub>4</sub>	472.3558	473.3622	-0.7	473.3622[M+H] <sup>+</sup> 455.3515[M+H-H <sub>2</sub> O] <sup>+</sup> 437.3521[M+H-2H <sub>2</sub> O] <sup>+</sup> 383.2943[M+H-H <sub>2</sub> O-C <sub>4</sub> H <sub>8</sub> O] <sup>+</sup>	e
92	23.93	Alisol L	468.3240	C <sub>30</sub> H <sub>44</sub> O <sub>4</sub>	468.3238	469.3310	-0.4	469.3310[M+H] <sup>+</sup> 451.3188[M+H-H <sub>2</sub> O] <sup>+</sup> 397.2745[M+H-C <sub>4</sub> H <sub>8</sub> O] <sup>+</sup>	e
93	24.47	Alisol O	512.3502	C <sub>32</sub> H <sub>48</sub> O <sub>5</sub>	512.3492	513.3581	0.3	513.3581[M+H] <sup>+</sup> 495.3475[M+H-H <sub>2</sub> O] <sup>+</sup> 453.3413[M+H-HAc] <sup>+</sup> 435.3628[M+H-HAc-H <sub>2</sub> O] <sup>+</sup>	e
94	24.65	Linolenic acid	278.2246	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278.2245	277.2164	-1.4	277.2164[M-H] <sup>-</sup>	a,d
95	25.28	Alisol A 24-acetate	532.3764	C <sub>32</sub> H <sub>52</sub> O <sub>6</sub>	532.3761	555.3748	1.5	555.3748[M+Na] <sup>+</sup> 515.3516[M+H-H <sub>2</sub> O] <sup>+</sup> 497.3472[M+H-2H <sub>2</sub> O] <sup>+</sup> 383.2797[M+H-C <sub>6</sub> H <sub>12</sub> O <sub>3</sub> -H <sub>2</sub> O] <sup>+</sup>	e

96 <sup>g</sup>	25.38	Alisol B 23-acetate	514.3658	C <sub>32</sub> H <sub>50</sub> O <sub>5</sub>	514.3661	537.3554	0.5	537.3554[M+Na] <sup>+</sup> 515.3735[M+H] <sup>+</sup> 497.3629[M+H-H <sub>2</sub> O] <sup>+</sup> 479.3508[M+H-2H <sub>2</sub> O] <sup>+</sup> 437.3415[M+H-H <sub>2</sub> O-HAc] <sup>+</sup> 383.2688[M+H-C <sub>6</sub> H <sub>12</sub> O <sub>3</sub> ] <sup>+</sup>	e
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Glc: glucose; Rha: rhamnose; Xyl: xylose; Res: resveratrol.

<sup>a</sup> *Rheum palmatum* L.

<sup>b</sup> *Coptis chinensis* Franch.

<sup>c</sup> *Curcuma longa* L.

<sup>d</sup> *Lysimachia christinae* Hance.

<sup>e</sup> *Alisma orientale* (Sam.) Juzep.

<sup>f</sup> *Panax notoginseng* (Burk.) F. H. Chen.

<sup>g</sup> Identified by comparison with reference standards.

terminal chain, such as -CH<sub>3</sub>. Peak 26 gave a protonated ion at  $m/z$  356.1865. The fragment ions at  $m/z$  192.1019 and 165.0893 were attributed to RDA cleavage at C- and B-rings. By further loss of a methyl radical, two obtained ions generated characteristic ions at  $m/z$  177.0774 and 150.0663, respectively. Therefore, peak 26 was tentatively identified as tetrahydropalmatine.

### 3.2.3. Protostane triterpenoids

17 protostane triterpenoids in GDD originated from *Alisma orientale* (Sam.) Juzep. Protostane triterpenoids showed [M+H]<sup>+</sup> ion, adduct [M+Na]<sup>+</sup> ion in positive ion mode and all possess a tetracyclic carbon skeleton. During the collision-induced dissociation (CID) process, the hydrogen rearrangement at C-23-OH resulting in C-23–C-24 bond dissociation was proposed as a characteristic CID fragmentation pathway, which can be used to further distinguish certain positional isomers containing the acetyl unit at the C-23 or C-24 position [23,24]. Such compounds usually occurred successive losses of H<sub>2</sub>O, acetic acid group (HAc, 60 Da) and other complex groups such as C<sub>4</sub>H<sub>8</sub>O (72 Da), C<sub>4</sub>H<sub>10</sub>O<sub>2</sub> (90 Da) and C<sub>6</sub>H<sub>12</sub>O<sub>3</sub> (132 Da). Peaks 96 was exactly identified as alisol B 23-acetate based on retention time and fragment behavior of reference standard. The high energy MS<sup>E</sup> spectra and the proposed fragment pathway of alisol B 23-acetate are depicted in Fig. 5. Alisol B 23-acetate showed [M+H]<sup>+</sup> and [M+Na]<sup>+</sup> ions at  $m/z$  515.3735 and 537.3554, which underwent several dehydrations or deacetylations to form fragment ions at  $m/z$  497.3629 [M + H-H<sub>2</sub>O]<sup>+</sup>, 479.3508 [M + H-2H<sub>2</sub>O]<sup>+</sup>, and 437.3415 [M + H-H<sub>2</sub>O-HAc]<sup>+</sup>, and then dissociation of the C-23–C-24 bond and loss of H<sub>2</sub>O gave rise to [M + H-C<sub>6</sub>H<sub>12</sub>O<sub>3</sub>]<sup>+</sup> ion at  $m/z$  383.2688. Peaks 87, 91, and 95 showed the similar fragmentation behavior to alisol B 23-acetate, and were identified as alisol A 23-acetate, alisol B, and alisol A 24-acetate, respectively.

Peak 92 had a protonated ion [M+H]<sup>+</sup> at  $m/z$  469.3310 with a molecular formula of C<sub>30</sub>H<sub>44</sub>O<sub>4</sub>, and formed characteristic ions at 451.3188 [M + H-H<sub>2</sub>O]<sup>+</sup> and  $m/z$  397.2745 [M + H-C<sub>4</sub>H<sub>8</sub>O]<sup>+</sup> through 23-OH dehydration and C-23–C-24 dissociation. Thus, it was assigned as Alisol L. Owing to similar cleavage patterns by loss of C<sub>4</sub>H<sub>8</sub>O, peaks 69 and 88 were deduced to be alisol C and 11-deoxy- Alisol C, respectively. Peak 62 exhibited [M+H]<sup>+</sup> ion at  $m/z$  505.3223. Three typical dehydration ions at  $m/z$  487.3327, 469.3285 and 451.3318 were generated from the hydroxyl groups. The dissociation of the C-23–C-24 bond via hydrogen rearrangement at C-23-OH produced diagnostic ion at  $m/z$  415.2824 [M + H-C<sub>4</sub>H<sub>10</sub>O<sub>2</sub>]<sup>+</sup>, with further loss of H<sub>2</sub>O generating an ion at  $m/z$  397.2783 [M + H-C<sub>4</sub>H<sub>10</sub>O<sub>2</sub>-H<sub>2</sub>O]<sup>+</sup>, so peak 62 was tentatively identified as 16-oxo- alisol A. Peak 90, 14Da less than that of 16-oxo- alisol A and similar to 16-oxo- alisol A, was further confirmed as alisol A.

### 3.2.4. Flavonoids

Ten flavones and their glycosides have been screened and identified in GDD using the UNIFI workflow. It is well known that the main MS behavior of flavone aglycones was RDA fragmentation pathway and losses of small molecules and/or radicals like CH<sub>3</sub>, CO and CO<sub>2</sub> [25]. For flavones glycosides, the cleavage at glycosidic linkages could happen in both positive and negative ion modes, and 162 Da (Glc), 146 Da (Rha) and 308 Da (rutinoside) were the characteristic neutral loss of flavonoid-O-glycosides. The fragment ions with low  $m/z$  were the same as that of their aglycones. Among them, peaks 43 and 71 were ascertained to be quercetin and kaempferide by contrast with reference standards. Here we took quercetin and kaempferide as examples to describe the fragment patterns of these components. Quercetin displayed a deprotonated ion at  $m/z$  301.0360 with a molecular formula of C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>, and the ions at  $m/z$  151.0451 [M-H-C<sub>8</sub>H<sub>6</sub>O<sub>3</sub>]<sup>-</sup>, 121.0431 [M-H-C<sub>8</sub>H<sub>6</sub>O<sub>3</sub>-

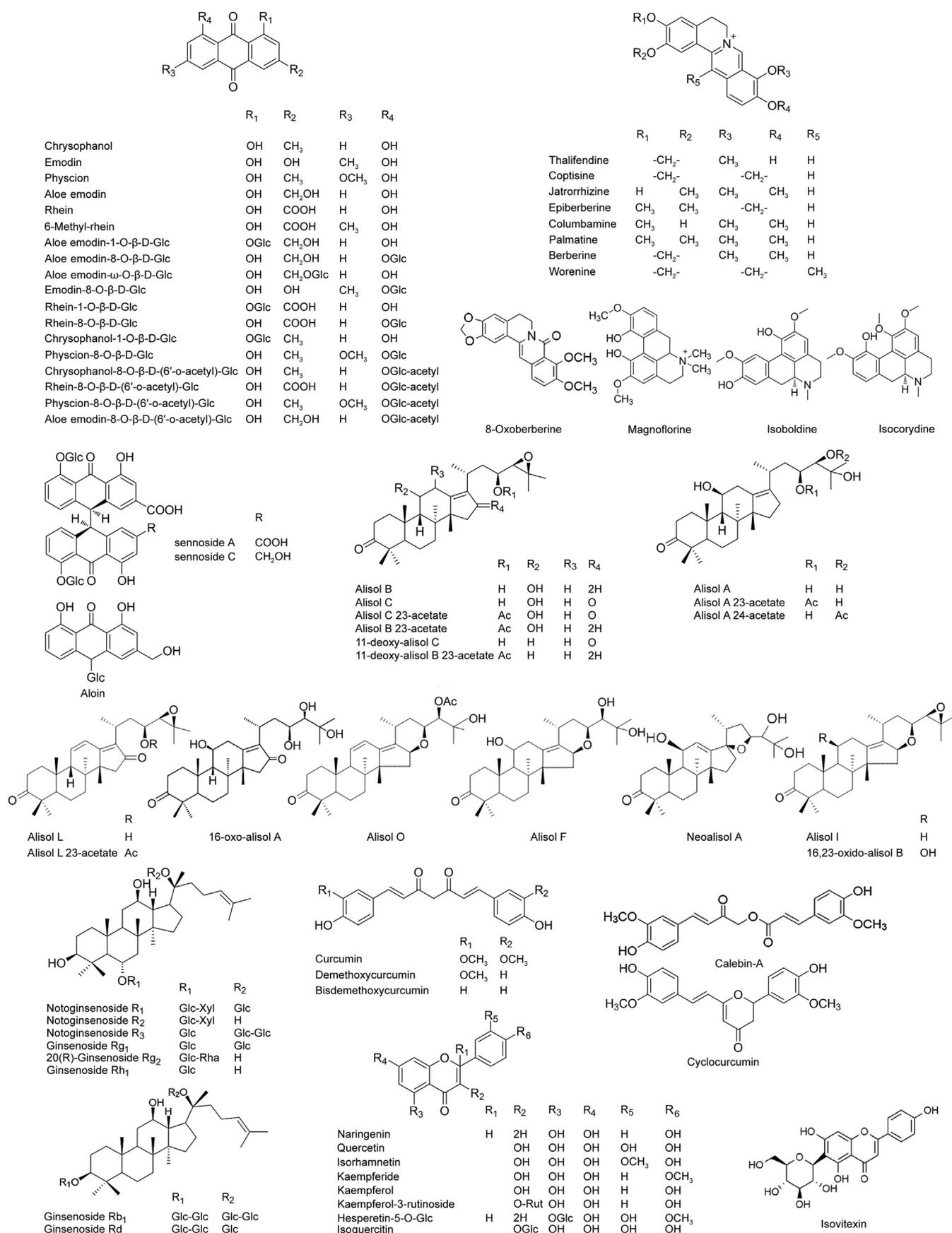


Fig. 2. Chemical structures of compounds identified in GDD.

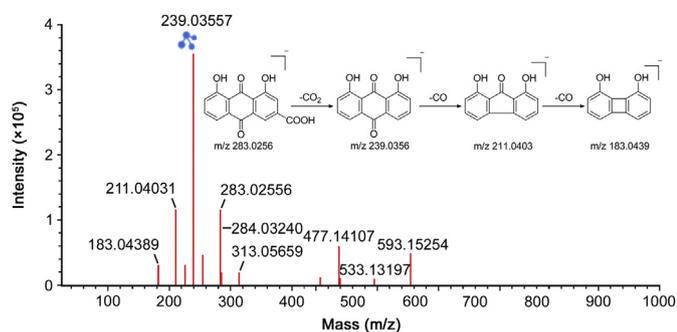


Fig. 3. The MS spectra and fragmentation pathway of rhein in negative ion mode.

$\text{CH}_2\text{O}]^-$  and  $107.0162 [\text{M}-\text{H}-\text{C}_8\text{H}_6\text{O}_3-\text{CH}_2\text{O}-\text{CH}_2]^-$  resulted from RDA cleavage. Kaempferide, with the parent ion  $[\text{M}-\text{H}]^-$  at  $m/z$  299.0553, exhibited a diagnostic ion  $[\text{M}-\text{H}-\text{CH}_3]^-$  at  $m/z$  284.0489 and RDA cleavage ion at  $m/z$  151.0055.

Peak 42 presented  $[\text{M}-\text{H}]^-$  ion at  $m/z$  285.0406, which was 14 Da less than that of kaempferide, showing the similar fragment pathways as kaempferide. It was presumed as kaempferol. Peak 23 displayed  $[\text{M}-\text{H}]^-$  ion at  $m/z$  593.1521 and produced predominant fragment ions at 447.1591  $[\text{M}-\text{H}-\text{Rha}]^-$ , 285.0832  $[\text{M}-\text{H}-\text{Rha}-\text{Glc}]^-$  due to successive losses of glycoside fragments. Meanwhile, the ion at  $m/z$  285.0832 further generated the characteristic ions identical to those of kaempferol, so the structure of this compound was considered as kaempferol-3-*O*-rutinoside. Analogously, peaks 16 and 30 were identified as rutin and hesperetin-5-*O*-glc, respectively.

### 3.2.5. Triterpenoid saponins

Eight triterpenoid saponins were detected from *Panax notoginseng* (Burk.) F. H. Chen in negative ion mode. These compounds offered the intense deprotonated ion  $[\text{M}-\text{H}]^-$  and adduct ion  $[\text{M} + \text{HCOO}]^-$ . The primary fragmentation pattern of triterpenoid saponins was the successive losses of glycosidic unit at the site of C-20, C-3 or C-6 of ginsenosides until the formation of  $[\text{Aglycon}-\text{H}]^-$  ions. The species and amount of glycosyl groups were observed from MS data, in which the mass differences of 162Da, 132Da and 146Da indicated the presence of glucose (Glc), xylose (Xyl), and rhamnose (Rha), respectively [26]. Peak 37 was definitely identified as notoginsenoside  $\text{R}_1$  with a reference standard. To facilitate characterization of these ginsenosides, the MS fragmentation pattern of notoginsenoside  $\text{R}_1$  is investigated in detail (Fig. 6). Notoginsenoside  $\text{R}_1$  gave  $[\text{M}-\text{H}]^-$  ion at  $m/z$  931.5297 and  $[\text{M} + \text{HCOO}]^-$  ion at  $m/z$  977.5354, along with three major fragment ions at  $m/z$  799.4856  $[\text{M}-\text{H}-\text{Xyl}]^-$ , 637.4314  $[\text{M}-\text{H}-\text{Xyl}-\text{Glc}]^-$ , and 475.3782  $[\text{M}-\text{H}-\text{Xyl}-2\text{Glc}]^-$  observed in high energy  $\text{MS}^E$  spectra. Peak 55 showed deprotonated ion  $[\text{M}-\text{H}]^-$  at  $m/z$  1107.5967 with a molecular formula of  $\text{C}_{54}\text{H}_{92}\text{O}_{23}$ . The fragment ion at  $m/z$  459.3824  $[\text{M}-\text{H}-4\text{Glc}]^-$  represented glycosidic cleavage by loss of four glucose residues. Hence, it was tentatively characterized as ginsenoside  $\text{Rb}_1$ .

Peak 41 displayed  $[\text{M}-\text{H}]^-$  and  $[\text{M} + \text{HCOO}]^-$  ions at  $m/z$  799.4863 and 845.4921, respectively, and produced fragment ion at  $m/z$  475.3784 by loss of two glucose residues. Thus, it was tentatively assigned to be ginsenoside  $\text{Rg}_1$ . Peak 54 showed deprotonated ion at  $m/z$  769.4768. The fragment ions at  $m/z$  637.4335  $[\text{M}-\text{H}-\text{Xyl}]^-$  and 475.3786  $[\text{M}-\text{H}-\text{Xyl}-\text{Glc}]^-$  corresponded to successive neutral losses of xylose residue and glucose residue, indicating that peak 54 was notoginsenoside  $\text{R}_2$ . Peak 57 gave  $[\text{M}-\text{H}]^-$

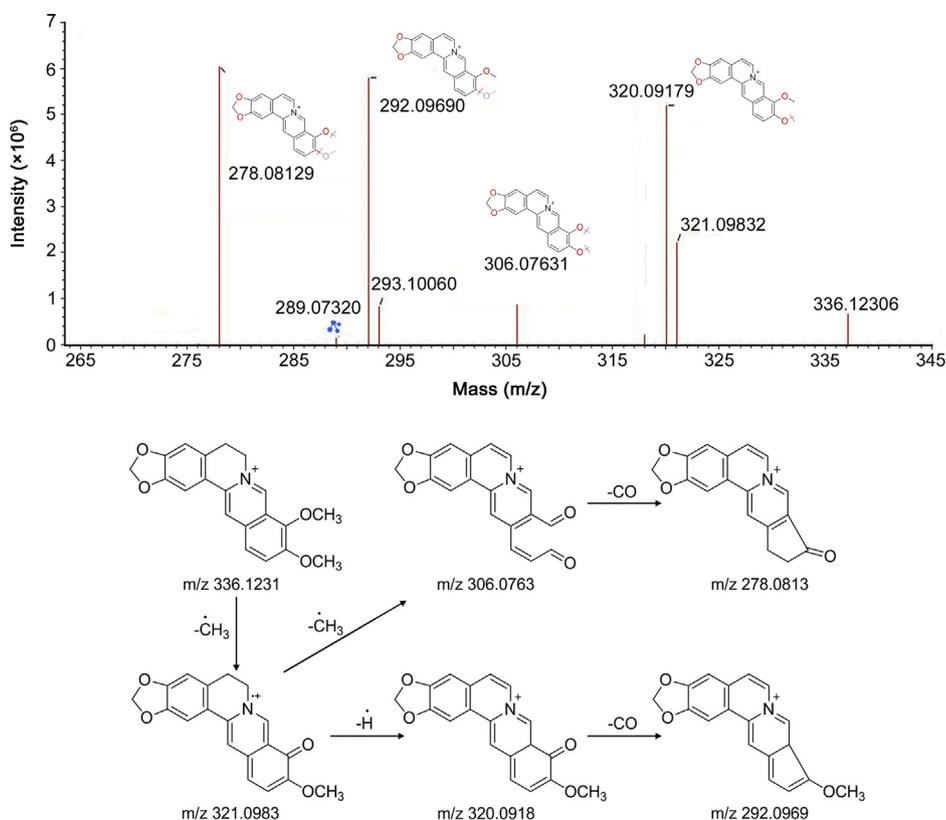


Fig. 4. The MS spectra and fragmentation pathway of berberine in positive ion mode.

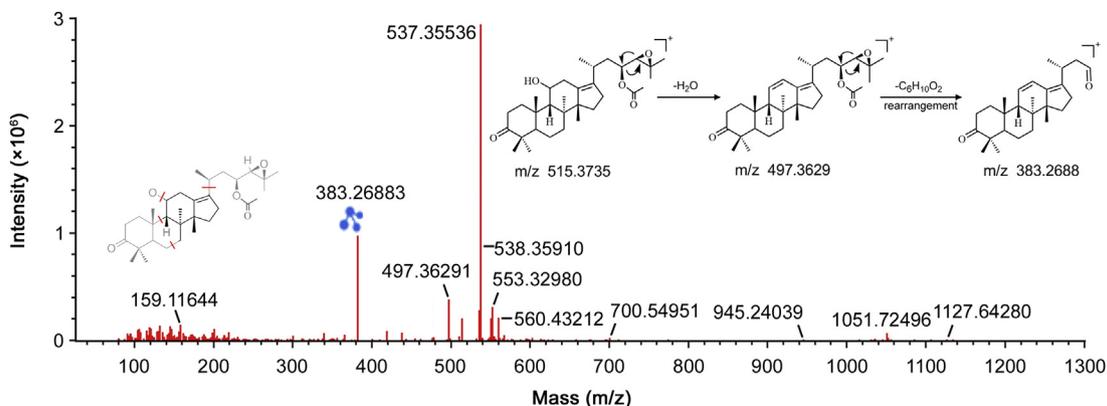


Fig. 5. The MS spectra and fragmentation pathway of alisol B 23-acetate in positive ion mode.

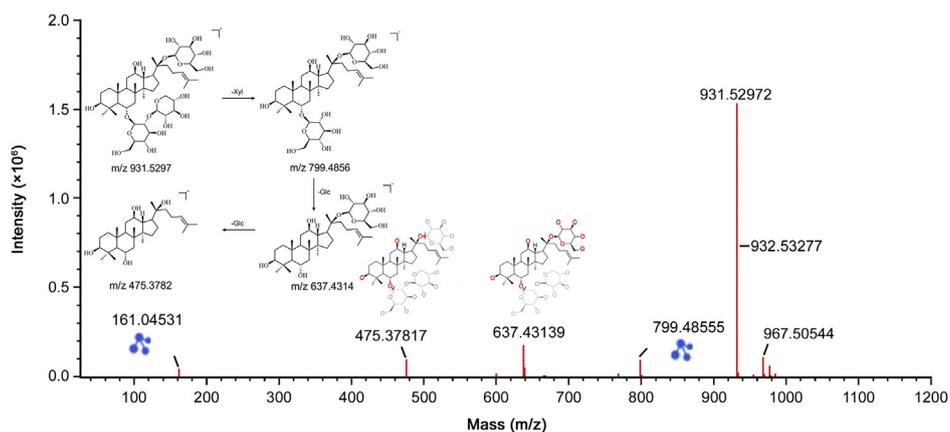


Fig. 6. The MS spectra and fragmentation pathway of notoginsenoside R<sub>1</sub> in negative ion mode.

at  $m/z$  783.4922, which further fragmented into  $m/z$  637.4326  $[M-H-Rha]^-$  and  $m/z$  475.3789  $[M-H-Rha-Glc]^-$ , so their fragment ions suggested that it was ginsenoside Rg<sub>2</sub>. According to the cleavage of glycosidic linkages discussed above, peaks 58, 63 and 66 were tentatively identified as ginsenoside Rh<sub>1</sub>, ginsenoside Rd, and notoginsenoside R<sub>3</sub>, respectively.

### 3.2.6. Others

Four curcuminoids were recognized as the major active components in *Curcuma longa* L. Peak 74 was unambiguously identified as curcumin by comparison with a reference standard. Curcumin was taken as an example, which gave precursor ion at  $m/z$  367.1192  $[M-H]^-$  and diagnostic ion at  $m/z$  217.0524  $[M-H-C_9H_{10}O_2]^-$  in negative ion mode. Analogously, peaks 56, 72 and 73 were identified as cyclocurcumin, bisdemethoxycurcumin and demethoxycurcumin, respectively. The fragmentation behaviors were in accordance with those previously reported in the literature [27].

In addition, there are small amounts of tannins in GDD, of which 10 components were identified as tannins or acylglucosides by comparing with the data in library. Peak 2 showed  $[M-H]^-$  ion at  $m/z$  169.0141 as base peak, and fragment ion of  $m/z$  125.0244 corresponding to the loss of CO<sub>2</sub> residues. So it was tentatively identified as gallic acid. Peak 5 gave  $[M-H]^-$  ion at  $m/z$  577.1359 with a molecule formula of C<sub>30</sub>H<sub>26</sub>O<sub>12</sub>, which yielded characteristic ions at  $m/z$  425.0873  $[M-H-C_7H_4O_4]^-$ , 407.0763  $[M-H-C_7H_4O_4-H_2O]^-$  and 289.0712  $[M-H-catechin]^-$ . Thus, it was tentatively deduced to be procyanidin B. Peak 9 exhibited  $[M-H]^-$  ion at  $m/z$  729.1471 and fragment ion at  $m/z$  577.1350  $[M-H-gallate]^-$ , suggesting that it

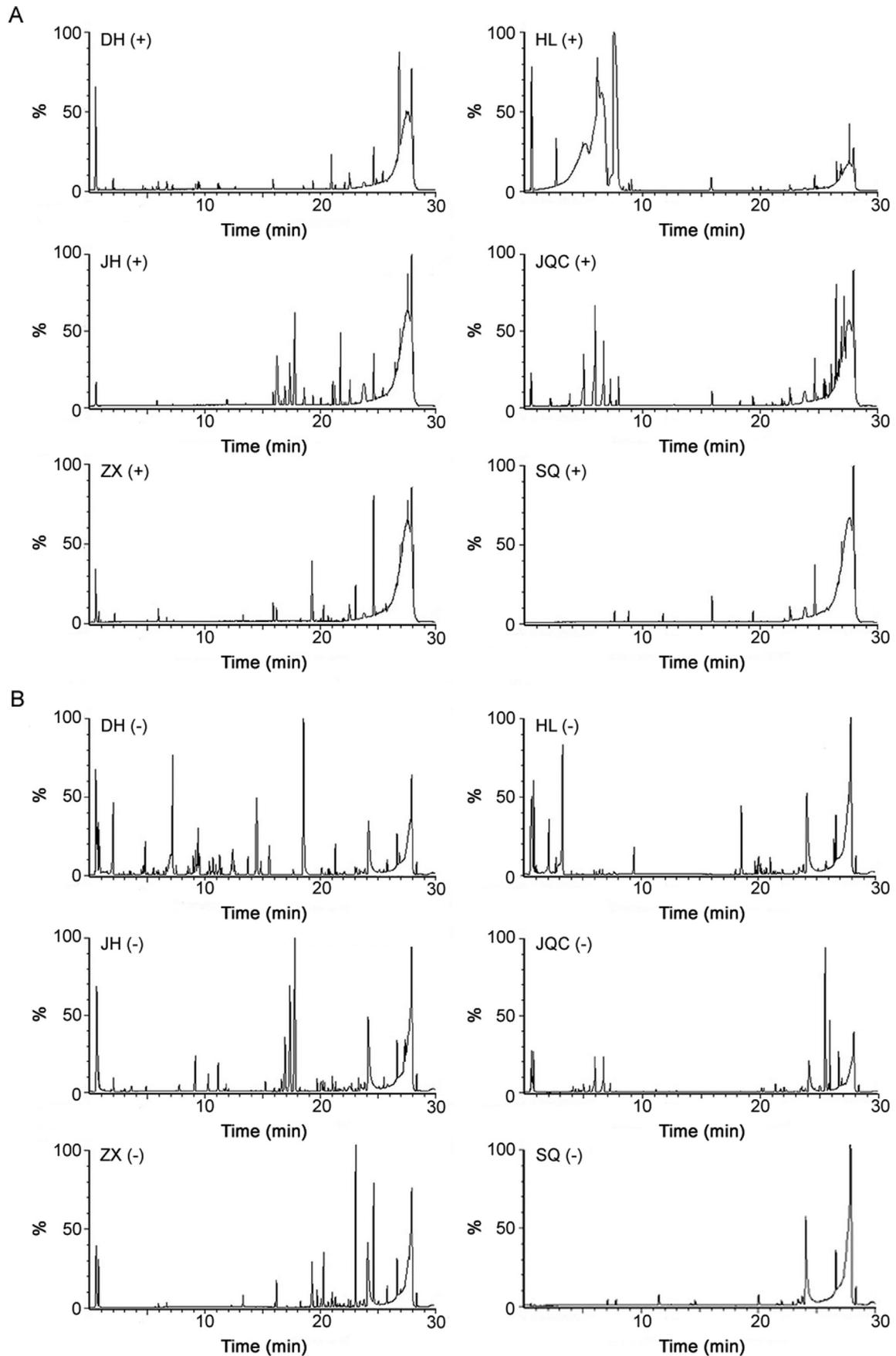
had an additional gallate than procyanidin B. It was tentatively identified as procyanidin B-O-gallate. Based on the similar fragment pattern, peaks 1, 4 and 19 were determined as gallic acid-O-glc, (+)-catechin-5-O-β-D-glc and (+)-catechin-3-O-gallate by matching the data in library, respectively.

### 3.3. Contribution of individual herbs to GDD

The established method was subsequently applied to analyze individual herbal decoctions by UPLC-Q-TOF-MS<sup>E</sup>, and the relative sources of 96 compounds were also correspondingly confirmed. In summary, 37 components were from *Rheum palmatum* L., 15 components were from *Coptis chinensis* Franch., 9 components came from *Curcuma longa* L., 15 components were from *Lysimachia christinae* Hance, 17 components were from *Alisma orientale* (Sam.) Juzep. and 8 triterpenoid saponins were from *Panax notoginseng* (Burk.) F. H. Chen. The BPI chromatograms of six individual herbs in positive and negative ion modes are shown in Fig. 7. But each individual herb undoubtedly contributed to chemical components in GDD. Therefore, different sources and multiple types of pharmacodynamic components can exert better therapeutic effect through synergism or complementation.

## 4. Conclusion

In this study, an integrative strategy based on UPLC-Q-TOF-MS<sup>E</sup> coupled with UNIFI informatics platform was applied for chemical profile analysis of GDD. To the best of our knowledge, it was the first



**Fig. 7.** The base peak intensity (BPI) chromatograms of six individual herbs in positive (A) and negative (B) ion modes. DH = *Rheum palmatum* L., HL = *Coptis chinensis* Franch., JH = *Curcuma longa* L., JQC = *Lysimachia christinae* Hance, ZX = *Alisma orientale* (Sam.) Juzep. and SQ = *Panax notoginseng* (Burk.) F. H. Chen.

time to reveal the constituents in GDD comprehensively. By comparison with retention time, accurate mass, fragmentation behavior, a total of 96 compounds were identified or tentatively characterized from GDD, including anthraquinones, alkaloids, protostane triterpenoids, flavonoids, triterpenoid saponins, tannins, curcuminoids and other compounds. Additionally, the ESI-MS fragmentation patterns of representative compounds in different chemical structure types were investigated. Most of the high response constitutions in individual herbs were also detected in GDD. This approach provided a rapid method for high throughput screening and characterization of constituents, and would be available in other TCM formulae analysis. What is more, the results could supply valuable information for the quality control and further study of GDD *in vivo*.

Moreover, we found that most of the compounds have abundant phenolic hydroxyl, especially the anthraquinones, curcumin and flavonoids. The structures of these compounds tend to be easily chelated by copper ions. Therefore, it is speculated that natural small molecules in GDD that could selectively chelate copper are able to form stable complexes to promote copper excretion. These molecules with properties would serve as a promising alternative to current treatments. This work has great guiding significance in further research and application of GDD in clinical treatment.

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#### Conflicts of interest

The authors declare that there are no conflicts of interest.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jpha.2019.05.003>.

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