Rapid identification of chemical profile in Gandou decoction by UPLC-Q-TOF-MS\textsuperscript{E} coupled with novel informatics UNIFI platform

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A B S T R A C T

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\textsuperscript{E}) 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, curcuminoinds, etc, were identified or tentatively characterized from GDD by comparing their retention time, accurate mass within 5 ppm error and MSE 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.

1. Introduction

Traditional Chinese medicines (TCM) have been extensively used for the prevention and treatment of complex and chronic diseases in China \cite{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 \cite{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 \cite{4,5}. Excessive copper accumulation in patients suffering from WD leads to liver disease, neurological disorder, K-F rings, and osteoporosis \cite{6}. Currently, there are several chelating agents such as d-penicillamine, dimercaptosuccinic acid, trientine, and tetrathiomolybdate for medical therapy \cite{7}. Although Western conventional medications are highly effective, prevalent, and low-priced, a number of side effects have been observed with chelation therapy \cite{8}. Gandou decoction (GDD), a classical TCM formula, has been used in clinics to treat WD for decades in China \cite{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 \cite{7,11}. Furthermore, GDD appears to be safe, effective, and well tolerated and has fewer adverse effects than Western conventional medications \cite{12}. In our previous studies, we investigated...
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® (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® capable of simultaneously acquiring accurate mass precursor ion in MS full scan and fragment ions in MS² 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® 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. Materials 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, physcion, emodin, alisol B 23-acetate, quercetin and notoginsenoside R₁, were obtained from the National Institutes for Food and Drug Control (Beijing, China). Chrysophanol, rhein, aloe-emodin and kaempferide were obtained from Beina Chuanqian 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 weigh have been identified by using several spectral analyses and MS, respectively. Acetonitrile and methanol (LC-MS grade) were purchased from Millipore (Fairfield, USA). Formic acid was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Ultra-pure 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 desicator 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 μL filtrate was injected into the UPLC-Q-TOF-MS® system for analysis after filtered through 0.22 μm 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 μm filter membrane.

2.3. Chromatography and mass spectrometry conditions

Chromatographic analysis was performed using a Waters Acquity™ 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 × 100 mm, 1.8 μm) 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%; 1–4 min, 10%–20%; 4–10 min, 20%–30%; 10–15 min, 30%–40%; 15–18 min, 40%–50%; 18–23 min, 50%–75%; 23–25 min, 75%–85%; 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⁺) or 110 °C (ESI⁻), cone gas flow of 50 L/h, desolvation gas (N₂) flow of 600 L/h and desolvation gas temperature of 350 °C. The collision voltage was set as 60 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 (Lock-Spray™) consisting of a 200 pg/mL solution of leucineenkephalin infused at a flow rate of 10 μL/min via a lockspray interface, generating a realtime reference ion of [M+H]+ (m/z 556.2771) in positive ion mode and [M−H]− (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™ 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 intensity of low energy over 200 counts were the selected parameters in 3D peak detection. A margin of error up to 5 ppm for 356 compounds is listed in Table S1.
containing HCOO⁻ and 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-MS⁵ 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, MS² 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-MS²

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, CH₃ and CO₂ [21,22]. Peaks 64, 65, 76, 83 and 86 were exactly identified as aloe 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 [M–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 CO₂ 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. Aloe emodin and emodin were isomers with the same [M–H⁻]⁻ ion at m/z 269. In high energy MS² spectra, emodin revealed [M–H⁻–CHO]⁻ ion at m/z 241.0507 and [M–H⁻–CO–O]⁻ ion at m/z 225.0559, while aloe emodin could be differentiated by the characteristic ion [M–H⁻–CHO]⁻ at m/z 240.0421. Physcion showed [M–H⁻]⁻ ion at m/z 283.0619, and the obvious fragments ions at m/z 255.0315 [M–H⁻–CHO]⁻ and 240.0359 [M–H⁻–CO–CH₃]⁻ were further obtained. Chrysophanol showed [M–H⁻]⁻ ion at m/z 253.0515, only one product ion at m/z 225.0545 [M–H⁻–CO].

For anthraquinone glycosides, aglycone ions were identified based on the MS fragmentation behaviors of free anthraquinones. Peak 47 exhibited [M–H⁻]⁻ ion at m/z 415.1045, which generated an [M–H⁻–162Da⁻]⁻ 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-β-D-glc. Based on these fragmentation patterns, peaks 15, 17, 32, 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 [M–H⁻]⁻ ion at m/z 561.1908, which first produced ions at m/z 699.1358 and 655.1880 by sequential loss of terminal glucose residue and CO₂, followed by the cleavage of C-10 and C-10’ forming [M–H⁻–GlC–CO₂–C₃H₇OH₂O₅⁻]⁻ 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 (CH₃·), 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 [M–CH₃]⁺, 320.0918 [M–CH₂–H]⁺, 306.0763 [M–2CH₃]⁺, 292.0969 [M–CH₂–H–CO]⁺ and 278.0813 [M–2CH₂–CO]⁺. Peak 40 showed [M⁺]⁺ ion at m/z 352.1538 and yielded characteristic ions at m/z 337.1430 [M–CH₃]⁺, 336.0983 [M–CH₂–H]⁺, 322.1068 [M–2CH₃]⁺, and 308.1275 [M–2CH₂–H–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, epiberberine, worenine and 8-oxoberberine, respectively.

Peak 10 gave [M⁺]⁺ ion at m/z 342.1701 with a molecular formula C₂₀H₂₄NO₄. The predominant ions appeared at m/z 297.1123 [M–(CH₃)₂NH]⁺, 282.0876 [M–(CH₃)₂NH–CH₃]⁺, 265.0861 [M–(CH₃)₂NH–CH₂OH]⁺, and 237.0908 [M–(CH₃)₂NH–CH₂–OH]⁺, 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
| No. | $t_R$ (min) | Identification                        | Formular     | Neutral mass (Da) | Observed neutral mass (Da) | Experimental mass (m/z) | Error (ppm) | MS and MSE data (+ or −) (m/z) | Source |
|-----|------------|---------------------------------------|--------------|------------------|---------------------------|-------------------------|-------------|--------------------------------|--------|
| 1   | 0.98       | Gallic acid-O-gluc                     | C$_{16}$H$_{16}$O$_{10}$ | 332.0743         | 332.0743                  | 331.0669                | −0.5        | 331.0669[M−H]$^-$               | a      |
| 2   | 1.05       | Gallic acid                           | C$_{16}$H$_{16}$O$_{5}$ | 170.0215         | 170.0214                  | 169.0141                | −0.8        | 169.0141[M−H]$^-$               | a      |
| 3   | 1.39       | Progallin A                            | C$_{9}$H$_{10}$O$_{5}$ | 198.0528         | 198.0528                  | 197.0455                | −0.7        | 197.0455[M−H]$^-$               | a      |
| 4   | 1.50       | (+)-Catechin-5-O-β-D-gluc             | C$_{21}$H$_{24}$O$_{11}$ | 452.1311         | 452.1318                  | 451.1245                | −0.7        | 451.1245[M−H]$^-$               | a      |
| 5   | 1.71       | Procyanidin B                          | C$_{30}$H$_{30}$O$_{12}$ | 578.1424         | 578.1432                  | 577.1359                | 1.4         | 577.1359[M−H]$^-$               | a      |
| 6   | 2.71       | Chlorogenic acid                       | C$_{16}$H$_{16}$O$_{5}$ | 354.0951         | 354.0962                  | 353.0889                | 3.1         | 353.0889[M−H]$^-$               | b,d    |
| 7   | 2.82       | (-)-Epicatechin                        | C$_{15}$H$_{16}$O$_{6}$ | 290.0790         | 290.0797                  | 289.0724                | 2.3         | 289.0724[M−H]$^-$               | a      |
| 8   | 3.16       | Isocorydine                            | C$_{20}$H$_{24}$O$_{10}$ | 341.1627         | 341.1628                  | 342.1707                | 0.1         | 342.1707[M−H]$^-$               | b      |
| 9   | 3.19       | Procyanidin B-O-gallate                | C$_{27}$H$_{30}$O$_{16}$ | 730.1534         | 730.1544                  | 729.1471                | 1.3         | 729.1471[M−H]$^-$               | a      |
| 10  | 3.20       | Magnoflorine                           | C$_{20}$H$_{24}$O$_{10}$ | 342.1705         | 342.1730                  | 342.1701                | −1.2        | 342.1701[M−H]$^-$               | b      |
| 11  | 3.42       | Isoboldine                             | C$_{19}$H$_{18}$O$_{5}$ | 322.1079         | 322.1055                  | 322.1052                | −6.6        | 322.1052[M−H]$^-$               | a      |
| 12  | 3.79       | Isovitexin                             | C$_{21}$H$_{24}$O$_{10}$ | 432.1056         | 432.1065                  | 477.1047                | 1.8         | 477.1047[M−HCOO]$^-$             | d      |
| 13  | 4.75       | Tetradehydroscoulerine                 | C$_{19}$H$_{18}$O$_{5}$ | 322.1079         | 322.1055                  | 322.1052                | −6.6        | 322.1052[M−H]$^-$               | b      |
| 14  | 4.79       | Procyanidin B-5,3,3’-di-O-gallate      | C$_{44}$H$_{34}$O$_{20}$ | 882.1643         | 882.1685                  | 881.1612                | 4.8         | 881.1612[M−H]$^-$               | a      |
| 15  | 4.86       | Emodin-8-O-β-D-gluc                   | C$_{21}$H$_{24}$O$_{10}$ | 432.1056         | 432.1065                  | 431.0992                | 1.9         | 431.0992[M−H]$^-$               | a      |
| 16  | 4.88       | Rutin                                 | C$_{19}$H$_{18}$O$_{16}$ | 610.1534         | 610.1555                  | 609.1483                | 3.6         | 609.1483[M−H]$^-$               | a,d    |
| 17  | 5.09       | Rhein-1-O-β-D-gluc                    | C$_{21}$H$_{24}$O$_{11}$ | 446.0849         | 446.0860                  | 445.0787                | 2.4         | 445.0787[M−H]$^-$               | a      |
| 18  | 5.11       | Isolindleyin or lindleyin             | C$_{22}$H$_{28}$O$_{11}$ | 478.1475         | 478.1483                  | 477.1410                | 1.7         | 477.1410[M−H]$^-$               | d      |
| 19  | 5.14       | (+)-Catechin-3-O-gallate              | C$_{22}$H$_{18}$O$_{10}$ | 442.0900         | 442.0910                  | 441.0838                | 2.4         | 441.0838[M−H]$^-$               | a      |
| No. | m/z | Retention Time (min) | Compound Name | Exact Mass (Da) | Molar Mass (Da) | Formula | Confirmation Level |
|-----|-----|---------------------|---------------|----------------|----------------|---------|-------------------|
| 20  | 5.20| Ferulic acid-\(\beta\)-D-glc | 356.1107 | 356.1113 | 355.1040 | \(\text{C}_{14}\text{H}_{18}\text{O}_{9}\) | 1.5 |
| 21  | 5.25| Isoquercitrin         | 464.0955 | 464.0972 | 463.0899 | \(\text{C}_{21}\text{H}_{20}\text{O}_{12}\) | 3.7 |
| 22  | 5.45| Isolindleyin or lindleyin | 478.1475 | 478.1482 | 477.1408 | \(\text{C}_{23}\text{H}_{20}\text{O}_{11}\) | 1.6 |
| 23  | 5.61| Kaempferol-3-O-rutinoside | 594.1585 | 594.1599 | 593.1521 | \(\text{C}_{27}\text{H}_{30}\text{O}_{15}\) | 1.6 |
| 24  | 5.65| Resveratrol-4’-O-\(\beta\)-D-(2’’-O-galloyl)-glc | 542.1424 | 542.1431 | 541.1333 | \(\text{C}_{23}\text{H}_{20}\text{O}_{12}\) | 1.2 |
| 25  | 5.70| Sennosite C or D     | 848.2164 | 848.2177 | 847.2104 | \(\text{C}_{42}\text{H}_{40}\text{O}_{19}\) | 1.5 |
| 26  | 5.89| Tetrahydropalmatine   | 355.1784 | 355.1792 | 356.1865 | \(\text{C}_{21}\text{H}_{20}\text{O}_{4}\) | 2.4 |
| 27  | 6.24| Sennoside A or B     | 862.1956 | 862.1981 | 861.1908 | \(\text{C}_{42}\text{H}_{36}\text{O}_{20}\) | 2.8 |
| 28  | 6.36| Homoorientin         | 448.1006 | 448.1000 | 447.0927 | \(\text{C}_{21}\text{H}_{20}\text{O}_{11}\) | -1.2 |
| 29  | 6.52| Thalifendine         | 322.1079 | 322.1069 | 322.1082 | \(\text{C}_{18}\text{H}_{20}\text{O}_{4}\) | -0.4 |
| 30  | 6.94| Hesperin-5-O-glc     | 464.1319 | 464.1313 | 463.1240 | \(\text{C}_{23}\text{H}_{20}\text{O}_{11}\) | 1.5 |
| 31  | 7.01| Coptisine            | 320.0923 | 320.0921 | 320.0921 | \(\text{C}_{18}\text{H}_{18}\text{O}_{4}\) | 0.6 |
| 32  | 7.08| Rhein-8-O-\(\beta\)-D-(6’’-O-acetyl)-glc | 488.0955 | 488.0949 | 487.0876 | \(\text{C}_{23}\text{H}_{20}\text{O}_{12}\) | 1.5 |
| 33  | 7.15| Jatrorrhizine        | 338.1392 | 338.1384 | 338.1384 | \(\text{C}_{20}\text{H}_{20}\text{O}_{4}\) | 2.4 |
| 34  | 7.35| Epiberberine         | 336.1236 | 336.1250 | 336.1250 | \(\text{C}_{20}\text{H}_{20}\text{O}_{4}\) | -4.2 |
| 35  | 7.47| Aloe emodin-8-O-\(\beta\)-D-glc | 432.1056 | 432.1068 | 431.0992 | \(\text{C}_{21}\text{H}_{22}\text{O}_{10}\) | 0.8 |
| 36  | 7.48| Tetrahydroberberine  | 339.1471 | 339.1466 | 340.1544 | \(\text{C}_{20}\text{H}_{22}\text{O}_{4}\) | -1.4 |
| 37  | 7.72| Notoginsenoside R1   | 932.5345 | 932.5372 | 977.5354 | \(\text{C}_{44}\text{H}_{36}\text{O}_{18}\) | 2.8 |
| 38  | 8.09| Worenine             | 334.1079 | 334.1080 | 334.1080 | \(\text{C}_{20}\text{H}_{20}\text{O}_{4}\) | -0.3 |
| 39  | 8.46| Berberine            | 336.1236 | 336.1230 | 336.1231 | \(\text{C}_{20}\text{H}_{20}\text{O}_{4}\) | 1.8 |
| 40  | 8.51| Palmatine            | 352.1549 | 352.1543 | 352.1538 | \(\text{C}_{21}\text{H}_{22}\text{O}_{4}\) | -1.7 |

(continued on next page)
| No. | tR (min) | Identification                        | Neutral mass (Da) | Formular        | Observed neutral mass (Da) | Experimental mass (m/z) | Error (ppm) | MS and MS² data (+ or −) (m/z) | Source |
|-----|---------|--------------------------------------|-------------------|----------------|--------------------------|-------------------------|-------------|--------------------------------|--------|
| 41  | 8.53    | Ginsenoside Rg₁                        | 800.4922          | C₄₂H₇₂O₁₄      | 800.4939                 | 845.4921                | 2.0         | 845.4921[M+HCOO]−                | f      |
|     |         |                                      |                   |                |                          |                         |             | 799.4863[M−H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | 637.4321[M−H-Glc]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 475.3784[M−H-2Glc]−             |        |
|     |         |                                      |                   |                |                          |                         |             | 285.0460[M−H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | 229.0451[M−H-C₂O₂]−             |        |
|     |         |                                      |                   |                |                          |                         |             | 169.0411[M−H-C₃O₃]−             |        |
|     |         |                                      |                   |                |                          |                         |             | 301.0360[M−H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | 151.0451[M−H-C₅H₉O₃]−           |        |
|     |         |                                      |                   |                |                          |                         |             | 121.0431[M−H-C₅H₉O₃-CH₂O]−      | a,d    |
|     |         |                                      |                   |                |                          |                         |             | 107.0162[M−H-C₅H₉O₃-CH₂O-CH₂]−   |        |
|     |         |                                      |                   |                |                          |                         |             | 417.1195[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 255.0666[M−H-Glc]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 227.0718[M−H-Glc-CO]−           | a      |
|     |         |                                      |                   |                |                          |                         |             | 431.0995[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 269.0452[M−H-Glc]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 225.0562[M−H-Glc-CO₂]−          |        |
|     |         |                                      |                   |                |                          |                         |             | 431.0995[M−H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | 269.0463[M−H-Glc]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 225.0560[M−H-Glc-CO₂]−          |        |
|     |         |                                      |                   |                |                          |                         |             | 415.1045[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 253.0515[M−H-Glc]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 225.0563[M−H-Glc-CO₂]−          |        |
|     |         |                                      |                   |                |                          |                         |             | 517.1003[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 473.1097[M−H-CO₂]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 269.0463[M−H-malonyl-Glc]−      |        |
|     |         |                                      |                   |                |                          |                         |             | 429.1193[M+HCOO]−               | a      |
|     |         |                                      |                   |                |                          |                         |             | 271.0616[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 151.0665[M−H-C₅H₉O₃]−           | d      |
|     |         |                                      |                   |                |                          |                         |             | 473.1096[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 269.0451[M−H-acetyl-Glc]−       |        |
|     |         |                                      |                   |                |                          |                         |             | 240.0410[M−H-acetyl-Glc-CHO]−   |        |
|     |         |                                      |                   |                |                          |                         |             | 445.1146[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 283.0617[M−H-Glc]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 240.0433[M−H-Glc-CHO]−          |        |
|     |         |                                      |                   |                |                          |                         |             | 457.1148[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 253.0511[M−H-acetyl-Glc]−       |        |
|     |         |                                      |                   |                |                          |                         |             | 225.0560[M−H-acetyl-Glc-CO₂]−    |        |
|     |         |                                      |                   |                |                          |                         |             | 815.4825[M+HCOO]−               |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 769.4768[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 637.4335[M−H-Xyl]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 475.3786[M−H-Xyl-Glc]−          |        |
|     |         |                                      |                   |                |                          |                         |             | 1107.5967[M−H]                 |        |
|     |         |                                      |                   |                |                          |                         |             | 945.5469[M−H-Glc]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 783.4917[M−H-2Glc]−             |        |
|     |         |                                      |                   |                |                          |                         |             | 621.4361[M−H-3Glc]−             |        |
|     |         |                                      |                   |                |                          |                         |             | 459.3824[M−H-4Glc]−             |        |
|     |         |                                      |                   |                |                          |                         |             | 367.1186[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 175.0400[M−H-C₅H₉O₃]−           | c      |
|     |         |                                      |                   |                |                          |                         |             | 829.4982[M+HCOO]−               |        |
|     |         |                                      |                   |                |                          |                         |             | 783.4922[M+H]                  |        |
|     |         |                                      |                   |                |                          |                         |             | [M+H]                          |        |
|     |         |                                      |                   |                |                          |                         |             | 637.4326[M−H-Rha]−              |        |
|     |         |                                      |                   |                |                          |                         |             | 475.3789[M−H-Rha-Glc]−          | f      |
| 56  | 12.69   | Cyclocurcumin                          | 368.1260          | C₂₁H₂₀O₆       | 368.1258                 | 367.1186                | -0.4        |                                | c      |
| 57  | 12.74   | Ginsenoside Rg₂                        | 784.4973          | C₄₂H₇₂O₁₃      | 784.4999                 | 829.4982                | 3.2         |                                | f      |
| No. | Retention Time | Compound Description                                      | Molar Ratio | Exact Mass (Da) | Relative Error | Molecule Formulas |
|-----|---------------|-----------------------------------------------------------|-------------|----------------|----------------|------------------|
| 58  | 12.94         | Ginsenoside Rh₁                                             |             | 638.4394       | 2.9            | C₃₆H₆₂O₉       |
| 59  | 13.18         | 6-Methyl-rhein                                              |             | 298.0477       | 1.1            | C₁₆H₁₀O₆       |
| 60  | 13.21         | Physcion-8-ß-D-(ß-D-6’-O-acyetyl)-glc                      |             | 488.1319       | 0.8            | C₂₄H₂₄O₁₁      |
| 61  | 13.51         | Orientaal A                                                 |             | 254.1882       | 8.3            | C₁₅H₂₂O₃       |
| 62  | 14.13         | 16-Oxo-alisol A                                             |             | 504.3451       | 4.3            | C₃₀H₄₈O₆       |
| 63  | 14.41         | Ginsenoside Rd                                              |             | 946.5501       | 8.0            | C₄₈H₈₂O₁₈      |
| 64  | 14.52         | Aloe emodin                                                  |             | 270.0528       | 3.0            | C₁₅H₁₀O₅       |
| 65  | 15.30         | Rhein                                                       |             | 284.0321       | 4.0            | C₁₅H₈O₆        |
| 66  | 16.01         | Notoginsenoside R₃                                          |             | 962.5450       | 4.2            | C₃₀H₄₆O₃       |
| 67  | 16.70         | 8-Oxoberberine                                               |             | 351.1107       | 0.4            | C₂₀H₁₇NO₅      |
| 68  | 16.72         | Alisol I                                                    |             | 454.3447       | 2.6            | C₉₀H₄₈O₃       |
| 69  | 17.05         | Alisol C                                                    |             | 486.3345       | 4.2            | C₃₀H₄₆O₅       |
| 70  | 17.24         | Ar-turmerone                                                 |             | 216.1514       | 0.7            | C₁₅H₂₀O     |
| 71  | 17.34         | Kaempferide                                                  |             | 300.0634       | -2.1           | C₁₄H₁₂O₆     |
| 72  | 17.46         | Bisdemethoxycurcumin                                         |             | 308.1049       | 2.9            | C₁₉H₁₆O₄     |
| 73  | 17.93         | Demethoxycurcumin                                            |             | 338.1154       | 2.0            | C₂₀H₁₆O₅     |
| 74  | 18.38         | Curcumin                                                    |             | 368.1260       | -0.1           | C₂₁H₂₀O₆     |
| 75  | 18.94         | Alisol M 23-acetate                                          |             | 488.3502       | -0.8           | C₉₀H₄₈O₃     |

(continued on next page)
| No. | 1s (min) | Identification               | Neutral mass (Da) | Formular               | Observed neutral mass (Da) | Experimental mass (m/z) | Error (ppm) | MS and MS² data (+ or −) (m/z) | Source |
|-----|----------|-------------------------------|-------------------|------------------------|-----------------------------|--------------------------|-------------|--------------------------------|--------|
| 76  | 19.25    | Emodin                        | 270.0528          | C₁₃H₁₂O₅               | 270.0533                    | 269.0460                 | 1.7         | 269.0460[M+H]+                  |        |
|     |          |                               |                   |                        |                             |                          |             | 241.0507[M+H-CO]−               |        |
|     |          |                               |                   |                        |                             |                          |             | 225.0539[M+H-CO-O]−             |        |
| 77  | 19.54    | Bisabolone oxide A            | 236.1776          | C₁₃H₂₀O₃               | 236.1776                    | 237.1850                 | 0.5         | 237.1850[M+H]+                  |        |
| 78  | 19.65    | Alisol C 23-acetate           | 528.3451          | C₁₅H₂₄O₆              | 528.3453                    | 529.3535                 | 1.0         | 529.3535[M+H]+                  |        |
|     |          |                               |                   |                        |                             |                          |             | 511.3415[M+H-CO]−               |        |
|     |          |                               |                   |                        |                             |                          |             | 469.3465[M+H-HAc]−              |        |
| 79  | 19.71    | Alisol F                      | 488.3502          | C₂₃H₂₄O₅              | 488.3497                    | 511.3391                 | −1.4        | 511.3391[M+Na]+                 |        |
| 80  | 20.04    | Alisol L 23-acetate           | 510.3345          | C₁₅H₂₄O₅              | 510.3530                    | 511.3418                 | 1.0         | 511.3418[M+H]+                  |        |
| 81  | 20.59    | Procyanidin                   | 594.1373          | C₁₇H₂₆O₁₃              | 594.1382                    | 593.1309                 | 0.8         | 593.1309[M+H]+                  |        |
| 82  | 21.39    | Neoalisol A                   | 488.3502          | C₁₅H₂₄O₆              | 488.3505                    | 489.3572                 | 2.5         | 489.3572[M+H]+                  |        |
| 83  | 21.71    | Chrysophanol                  | 254.0579          | C₁₃H₂₀O₄              | 254.0579                    | 253.0515                 | 3.3         | 253.0515[M+H]+                  |        |
| 84  | 21.72    | 16,23-Oxido alisol B          | 470.3396          | C₁₅H₂₄O₅              | 470.3395                    | 493.3287                 | −0.2        | 493.3287[M+H]+                  |        |
| 85  | 22.56    | Gingerdione                   | 292.1675          | C₁₇H₂₄O₄              | 292.1679                    | 337.1661                 | 1.4         | 337.1661[M+HCOO]−               |        |
| 86  | 22.80    | Physcion                      | 284.0685          | C₁₃H₁₂O₃              | 284.0692                    | 283.0619                 | 2.5         | 283.0619[M+H]+                  |        |
| 87  | 22.90    | Alisol A 23-acetate           | 532.3764          | C₁₅H₂₄O₆              | 532.3767                    | 555.3749                 | 0.6         | 555.3749[M+H]+                  |        |
| 88  | 23.05    | 11-Deoxy-alisol C             | 470.3396          | C₁₅H₂₄O₆              | 470.3398                    | 471.3380                 | 0.3         | 471.3380[M+H]+                  |        |
| 89  | 23.32    | Stearic acid                  | 284.2715          | C₁₇H₃₂O₂              | 284.2717                    | 283.2645                 | 0.7         | 283.2645[M+H]+                  |        |
| 90  | 23.54    | Alisol A                      | 490.3658          | C₁₅H₂₄O₅              | 490.3678                    | 491.3661                 | 3.8         | 491.3661[M+H]+                  |        |
| 91  | 23.79    | Alisol B                      | 472.3553          | C₁₅H₂₄O₆              | 472.3558                    | 473.3622                 | −0.7        | 473.3622[M+H]+                  |        |
| 92  | 23.93    | Alisol L                      | 468.3240          | C₁₅H₂₄O₆              | 468.3238                    | 469.3310                 | −0.4        | 469.3310[M+H]+                  |        |
| 93  | 24.47    | Alisol O                      | 512.3502          | C₁₅H₂₄O₅              | 512.3492                    | 513.3581                 | 0.3         | 513.3581[M+H]+                  |        |
| 94  | 24.65    | Linolenic acid                | 278.2246          | C₁₅H₂₄O₂              | 278.2245                    | 277.2164                 | −1.4        | 277.2164[M+H]+                  |        |
| 95  | 25.28    | Alisol A 24-acetate           | 532.3764          | C₁₅H₂₄O₅              | 532.3761                    | 555.3748                 | 1.5         | 555.3748[M+Na]+                 |        |
|     |          |                               |                   |                        |                             |                          |             | 515.3516[M+H-CO]−               |        |
|     |          |                               |                   |                        |                             |                          |             | 497.3472[M+H-HAc]−              |        |
|     |          |                               |                   |                        |                             |                          |             | 383.2797[M+H-CO-O₂·H₂O]−        |        |
terminal chain, such as –CH₃. 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]+ ion, adduct [M+Na]+ 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 H₂O, acetic acid group (HAc, 60 Da) and other complex groups such as C₆H₄O₂ (72 Da), C₆H₁₀O₂ (90 Da) and C₆H₁₂O₃ (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 MS² spectra and the proposed fragment pathway of alisol B 23-acetate are depicted in Fig. 2. Alisol B 23-acetate showed [M+Na]+ and [M+H]+ 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 + H₂O]+, 479.3508 [M + H₂O-HAc]+, and 437.3415 [M + H₂O-H₄O]+, and dissociation of the C-23—C-24 bond and loss of H₂O gave rise to [M + H-C₆H₄O₃]+ 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]+ at m/z 469.3310 with a molecular formula of C₁₅H₁₀O₇, and formed characteristic ions at 451.3188 [M + H₂O]+ and m/z 397.2745 [M + H-C₄H₆O₃]+ 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 C₆H₄O₂, peaks 69 and 88 were deduced to be alisol C and 11-deoxy-Alisol C, respectively. Peak 62 exhibited [M+H]+ 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-C₆H₁₀O₂]+, with further loss of H₂O generating an ion at m/z 397.2783 [M + H-C₆H₁₀O₂-H₂O]+, 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 CH₃, CO and CO₂ [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 C₁₅H₁₀O₇, and the ions at m/z 151.0451 [M−H-C₆H₄O₃]−, 121.0431 [M−H-C₆H₄O₃−

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References:

23. Xu, L.; et al. *Journal of Pharmaceutical Analysis* 10 (2020) 35–48
Fig. 2. Chemical structures of compounds identified in GDD.
CH₂O and 107.0162 [M–C₆H₄O₃–CH₂O–CH₂] resulted from RDA cleavage. Kaempferide, with the parent ion [M–H] at m/z 299.0553, exhibited a diagnostic ion [M–H–CH₃] at m/z 284.0489 and RDA cleavage ion at m/z 151.0055.

Peak 42 presented [M–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 [M–H] ion at m/z 593.1521 and produced predominant fragment ions at 447.1591 [M–H–Xyl], 285.0832 [M–H–Xyl–Glc] due to successive losses of glycosidic 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 [M–H]− and adduct ion [M + 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 [Aglycon–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 R₁ with a reference standard. To facilitate characterization of these ginsenosides, the MS fragmentation pattern of notoginsenoside R₁ is investigated in detail (Fig. 6). Notoginsenoside R₁ gave [M–H]− ion at m/z 931.5297 and [M + HCOO]− ion at m/z 977.5354, along with three major fragment ions at m/z 799.4856 [M–H–Xyl], 637.4314 [M–H–Xyl–Glc], and 475.3782 [M–H–Xyl–2Glc] observed in high energy MS² spectra. Peak 55 showed deprotonated ion [M–H]− at m/z 1107.5967 with a molecular formula of C₅₄H₉₂O₂₃. The fragment ion at m/z 459.3824 [M–H–4Glc]− represented glycosidic cleavage by loss of four glucose residues. Hence, it was tentatively characterized as ginsenoside Rb₁.

Peak 41 displayed [M–H]− and [M + 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 Rg₁. Peak 54 showed deprotonated ion at m/z 769.4768. The fragment ions at m/z 637.4335 [M–H–Xyl] and 475.3786 [M–H–Xyl–Glc] corresponded to successive neutral losses of xylose residue and glucose residue, indicating that peak 54 was notoginsenoside R₂. Peak 57 gave [M–H]−
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 Rg2. According to the cleavage of glycosidic linkages discussed above, peaks 58, 63 and 66 were tentatively identified as ginsenoside Rh1, ginsenoside Rd, and notoginsenoside R3, 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-C4H8O2]– 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 CO2 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 C30H26O12, which yielded characteristic ions at m/z 425.0873 [M–H-C6H4O2]–, 407.0763 [M–H-C6H4O2-H2O]– 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-MS², 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-MS² 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, tanins, 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 constituents 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.

Acknowledgments
This research was supported by the National Natural Science Foundation of China (No: 81872976, 81873038, 81303245), Key Project of the National Science Fund of Anhui Province (No: KJ2016A398), Key Project Foundation of Oversea Visiting and Research for the Excellent Young and Middle-aged Faculties in Universities of Anhui Province (No: gxgwfx20180402), and Primary Research & Development Plan of Anhui Province (No: 201904a07020051).

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.

References
[1] R. Stone, Lifting the veil on traditional Chinese medicine, Science 319 (2008) 709–710.
[2] F. Cheung, TCM: made in China, Nature 480 (2011) S82–S83.
[3] X.L. Zhao, X.Z. Zhang, L.Q. Di, et al., Exploration of thoughts and methods in study on material base of traditional Chinese medicinal herbs prescriptions, World Sci. Technol. 11 (2000) 488–492.
[4] A. Ala, A.P. Walker, K. Ashkan, et al., Wilson’s disease, Lancet 369 (2007) 397–408.
[5] M.L. Schilsky, A century for progress in the diagnosis of Wilson disease, J. Trace Elem. Med. Biol. 28 (2014) 492–494.
[6] E.A. Roberts, M.L. Schilsky, Diagnosis and treatment of Wilson disease: an update. Hepatology 47 (2008) 2089–2111.
[7] J. Liu, J. Luan, X. Zhou, et al., Epidemiology, diagnosis, and treatment of Wilson’s disease, Intractable Rare Dis. Res. 6 (2017) 249–255.
[8] W.J. Li, C. Chen, Z.F. You, et al., Current drug managements of Wilson’s disease: from west to east, Curr. Neuropharmacol. 14 (2016) 322–325.
[9] B.C. Xue, R.M. Yang, J.Y. Hu, Effect of Gandou Decoction IV combined with short-term decoper therapy with sodium dimercapto-sulphonate on serum indexes of hepatic fibrosis in patients with Wilson’s disease, Chin. J. Intern. Med. 27 (2007) 785–788.
[10] J. Zhang, J.Y. Hu, X.F. Ma, et al., Effect of Gandou Decoction combined with decoper therapy on quality of life in patients with Wilson’s disease: a prospective study of 135 cases, J. Anhui Univ. Chin. Med. 34 (2015) 14–16.
[11] Y. Liu, M.M. Cheng, J.J. Dong, et al., Intestinal absorptive characteristics of ingredients from ethanol extracts of Gandou decoction by rat ized intestina sac models, China J. Chin. Mater. Med. 42 (2017) 2571–2576.
[12] Y. Wang, C.L. Xie, D.L. Fu, et al., Clinical efficacy and safety of Chinese herbal medicine for Wilson’s disease: a systematic review of 9 randomized controlled trials, Complement. Ther. Med. 20 (2012) 143–154.
[13] M. Cheng, H. Wu, H. Wu, et al., Metabolic profiling of copper-laden Hepatotencular degeneration model rats and the interventional effects of Gandou Decoction using UPLC-Q-TOF/MS, J. Pharm. Biomed. Anal. 164 (2019) 187–195.
[14] C.J. Wang, Y.Q. Jiang, D.H. Liu, et al., Characterization of phloroglucinol derivatives and diterpenes in Euphorbia ebrickateau Hayata by utilizing ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry, J. Pharm. Anal. 3 (2013) 292–297.
[15] L. Bao, X. Bao, P. Li, et al., Chemical profiling of Malva verticillata L. by UPLC-Q-TOF MS® and their antioxidant activity in vitro, J. Pharm. Biomed. Anal. 150 (2018) 420–426.
[16] M. Fan, K. Qin, F. Ding, et al., Identification and differentiation of major components in three different “Sheng-ma” crude drug species by UPLC/Q-TOF-MS, Acta Pharm. Sin. B 7 (2017) 185–192.
[17] L. Deng, A.M. Shi, H.Z. Liu, et al., Identification of chemical ingredients of peanut stems and leaves extracts using UPLC-QTOF-MS coupled with novel informatics UNIFI platform, J. Mass Spectrom. 51 (2016) 1157–1167.
[18] P.P. Liu, G.S. Shan, F. Zhang, et al., Metabolomics analysis and rapid identification of changes in chemical ingredients in crude and processed Astragalus Radix by UPLC-QTOF-MS combined with novel informatics UNIFI platform, Chin. J. Nat. Med. 16 (2018) 714–720.
[19] X. Gao, Y. Ma, Z. Wang, et al., Identification of anti-inflammatory active ingredients from Tumuxiang by ultra-performance liquid chromatography/quadrupole time-of-flight MS®, Biomed. Chromatogr. 32 (2018) e1719.
[20] J.F. Tang, W.X. Li, X.J. Tan, et al., A novel and improved UHPLC-QTOF/MS method for the rapid analysis of the chemical constituents of Danhong injection, Anal. Methods 8 (2016) 2904–2914.
[21] H. Zhu, R. Yin, F. Han, et al., Characterization of chemical constituents in Zhi-Zi-Da-Huang decoction by ultra high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry, J. Sep. Sci. 37 (2014) 3489–3496.
[22] H.Y. Zhao, M.X. Fan, X. Wu, et al., Chemical profiling of the Chinese herb formula Xiao-Cheng-Qi Decoction using liquid chromatography coupled with electrospray ionization mass spectrometry, J. Chromatogr. Sci. 51 (2013) 273–285.
[23] Q. Liu, Z. Jao, Y. Liu, et al., Chemical profiling of San-Huang decoction by UPLC-ESI-Q-TOF-MS, J. Pharm. Biomed. Anal. 131 (2016) 20–32.
[24] W. Zhao, X. Huang, X. Li, et al., Qualitative and quantitative analysis of major triterpenoids in Alismatis Rhizoma by high performance liquid chromatography/diode-array detector/quadrupole-time-of-flight mass spectrometry and ultra-performance liquid chromatography/triple quadrupole mass spectrometry, Molecules 20 (2015) 13958–13981.
[25] S. Wang, L. Chen, J. Long, et al., Fragment ion diagnostic strategies for the comprehensive identification of chemical profile of Gui-Zhi-Tang by integrating high-resolution MS, multiple-stage MS and UV information, J. Pharm. Biomed. Anal. 98 (2014) 22–35.
[26] L. Xu, L.H. Mu, J. Peng, et al., UPLC-Q-TOF-MS® analysis of the constituents of Ding-Zhi-Xiao-Wan, a traditional Chinese antidepressant, in normal and depressive rats, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1026 (2016) 36–42.
[27] K. Ashraf, M. Mujeeb, A. Ahmad, et al., Determination of curcuminoids in Curcuma longa Linn. by UPLC/Q-TOF-MS: an application in turmeric cultivation, J. Chromatogr. Sci. 53 (2015) 1346–1352.