The Combination of UHPLC-HRMS and Molecular Networking Improving Discovery Efficiency of Chemical Components in Chinese Classical Formula

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Research Article

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Abstract

Background

It is essential to identify the chemical components for the quality control methods establishment of Chinese Classical Formula (CCF). However, CCF are complex mixture of several herbal medicines with huge number of different compounds and they are not equal to the combination of chemical components from each herb due to particular formula ratio and preparation techniques. Therefore, it is time-consuming to identify compounds in a CCF by analyzing the LC-MS/MS data one by one, especially for unknown components.

Methods

An ultra-high pressure liquid chromatography-linear ion trap-orbitrap high resolution mass spectrometry (UHPLC-LTQ-Orbitrap-MS/MS) approach was developed to comprehensively profile and characterize multi-components in CCF with Erdong decoction composed of eight herbal medicines as an example. Then the MS data of Erdong decoction was analyzed by MS/MS-based molecular networking and these compounds with similar structures were connected to each other into a cluster in the network map. Then the unknown compounds connected to known compounds in a cluster of the network map were identified due to their similar structures.

Results

Based on the clusters in the molecular networking, 113 compounds were rapidly identified from Erdong decoction for the first time, which including steroidal saponins, triterpenoid saponins, flavone O-glycosides and flavone C-glycosides.

Conclusion

MS/MS-based molecular networking technique is very useful for the rapid identification of components in CCF. In Erdong decoction, this method was very suitable for the identification of major steroidal saponins, triterpenoid saponins, and flavone C-glycosides.

Background

The Chinese Classical Formula (CCF) are the essences of thousands of years of practical experience in the clinical application of tradition Chinese medicines (TCM). It is important and preferred direction of traditional Chinese medicine (TCM) to develop CCF into modern preparations to meet the needs of convenience. The chemical components analysis is of great significance for the study of pharmacologically active components and the establishment of quality control methods of CCF. The main chemical components of CCF are extremely complex and they are not equal to the combination of chemical components of each herb due to different formula proportions and preparation techniques. Therefore, how to quickly identify the main chemical components of a TCM formula is an important step for the modernization development of CCF.

Identification of chemical components of TCM formula have been facilitated by modern analytical techniques. In particular, high-resolution mass spectrometry (HRMS) plays a critical role in characterizing structures of chemical compounds by providing precise molecular weight as well as fragmental structures with the advantages of high sensitivity and throughput in detecting versatile molecules [1]. Conventionally, liquid chromatography mass spectrometry (LC-MS) is one of the most widely used approaches to the preliminary characterization of chemical components of TCM formula extract. Nevertheless, it is time-consuming and difficult to analyze the MS data of a TCM formula due to its complex components, especially for unknown components.

Recently, the combination of LC-HRMS and molecular networking has facilitated the MS data analysis. Molecular networking (MN) is outstanding to dispose of complicated MS data. It is capable of gathering the molecules with similar structures together based on the similarity of their MS/MS fragments. Compounds that share similar MS/MS fragmentation patterns or molecular classes are likely to group together in MN. This improves the possibility of identification of unidentified nodes, if their spectra or the spectra of surrounding nodes are known by references [2–4]. Thus, the combination of LC-HRMS and molecular networking immensely enhances the efficiency and drastically reduces the time on data processing. In the last few decades, molecular networking was introduced in drug development and metabolomics, particularly for natural products containing hundreds of components.

As one example from the "Catalogue of Ancient Chinese Classic formula (First Batch)", Erdong decoction was record in yixuexinwu and used in nourishing Yin and quenching thirst. In modern clinical practice, Erdong decoction and its modified prescriptions have been mainly used to treat type 2 diabetes and its complications [5, 6]. It was composed of eight herbs including Asparagi Radix (the radix of Asparagus cochinchinensis(Lour.)Merr.), Ophiopogonis Radix (the radix of Ophiopogon japonicus.), Trichosanthis Radix (the radix of Trichosanthes kirilowii Maxim.), Scutellariae Radix (the radix of Scutellaria baicalensis Georgi.), Anemarrhe Naerhizoma (the naerhizoma of Anemarrhena asphodeloides Bunge.), Glycyrrhizae Radix Et Rhizoma (the radix et rhizoma of Glycyrrhiza uralensis Fisch.), Ginseng Radix Et Rhizoma (the radix et rhizoma of Panax ginseng C. A. Mey.) and
Nelumbinis Folium (the folium of *Nelumbo nucifera* Gaertn.). However, hitherto there is no report on systematic characterization of chemical components of Erdong decoction and its quality control methods.

In this study, the combination of LC-HRMS and molecular networking was applied to rapidly identify compounds in Erdong decoction as a case study to demonstrate the application of the combined techniques in TCM formula. An ultra-high pressure liquid chromatography-linear ion trap-orbitrap high resolution mass spectrometry (UHPLC-LTQ-Orbitrap-MS/MS) approach was developed to comprehensively profile and characterize multi-components in Erdong decoction. Then the MS data of Erdong decoction was analyzed by MS/MS-based molecular networking (Fig. 1). The results show that the combination of LC-HRMS and molecular networking greatly improves the efficiency of chemical components identification in CCF.

**Materials And Methods**

**Materials and reagents**

*A. cochinchinensis* was purchased from Guizhou Province in July 2018. *O. japonicus* was purchased from Santai, Sichuan Province in July 2018. *T. kirilowii* was purchased from Feicheng, Shandong Province in July 2018. *S. baicalensis* was purchased from Lingchuan, Shanxi Province in July 2018. *A. asphodeloides* was purchased from Wanrong, Shanxi Province in July 2018. *G. uralensis* was purchased from Beitun Town, Xinjiang Province in July 2018. *P. ginseng* was purchased from Fushong, Jilin Province in July 2018. *N. nucifera* was purchased from Nanchang, Jiangxi Province in September 2018. Reference compounds, neomangiferin, oroxylin A-7-O-β-D-glucuronide and glycyrrhizin acid were purchased from Beijing Century Aoko Biotechnology Co. Ltd. (Beijing, China), mangiferin, baicalin and wogonoside were purchased from National Institutes for Food and Drug Control (Beijing, China), and quercetin-3-O-gluconuride and hyperoside were purchased from Chengdu Cloma Biological Technology Co. Ltd. (Sichuan, China). HPLC-grade acetonitrile and LC-MS-grade formic acid were purchased from Fisher Scientific (USA).

**Sample Preparation**

The solutions of neomangiferin, mangiferin, hyperoside, quercetin-3-O-gluconuride, baicalin, oroxylin A-7-O-β-D-glucuronide, wogonoside and glycyrrhizic acid were prepared in methanol at appropriate concentrations. A mixture of 8 different slices consisting of 33.6 g of dried *O. japonicus* radixs, 22.5 g of dried *A. cochinchinensis* radixs, 11.1 g of dried *T. kirilowii* radixs, 11.1 g of dried *S. baicalensis* radixs, 11.1 g of dried *A. asphodeloides* naerhizomas, 11.1 g of dried *N. nucifera* foliums, 5.7 g of dried *G. uralensis* radix et rhizoma, and 5.7 g of dried *P. ginseng* radix et rhizome were extracted twice with water for 40 min, with ten times (w/v) water and six times (w/v) water, respectively. All extraction solutions were concentrated to 560 mL at 60°C. One hundred microlitre of concentrated solution was dissolved in 900 µL of 10% acetonitrile and centrifuged at 13000 r·min⁻¹ for 5 min, then the supernatant solution was filtered through a 0.22 µm membrane filter prior to injection into the chromatographic system.

**Data Acquisition And Molecular Networking Analysis**

HPLC analysis was performed on Dionex Ultimate 3000 UHPLC system (USA) with photodiode array (PDA) detector. Samples were separated on an Acquity UPLC HSS T3 column (100 × 2.1 mm i.d., 1.8µm) at 40°C. The mobile phase consisted of acetonitrile (A) and water containing 0.1% formic acid (B). A gradient program was adopted as follows: 0-3min, 10–13%; 3-6min, 13–14% A; 6-9min, 14–17% A; 9-11min, 17–25% A; 11-18min, 25–30% A; 18-19min, 30–48% A; 19-22min, 48–48% A, with a flow rate of 0.4 mL/min. The PDA detector scanned at 254 nm.

The LTQ-Orbitrap XL mass spectrometer was purchased from Thermo Scientific equipped with electrospray ionization (EIS) and Xcalibur 2.1 workstation. The analysis was performed in both negative and positive mode with a mass range of m/z 100–1400. High-purity nitrogen (N₂) was used as auxiliary gas (10 arb) and sheath gas (40 arb). The other parameters were as follows: capillary temperature, 350°C; capillary voltage, 3.3 kV (in the positive mode), 3.0 kV (in the negative mode).

The MS data of the targeted fraction was converted from the raw format to the mzXML format using the Proteo-Wizard 3.0.20014. Then, the mzXML file was uploaded by the suggested software of WinSCP (https://winscp.net/eng/download.php) to the GNPS platform (https://gnps.ucsd.edu). The resulting analysis and parameters for the network can be accessed via links http://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=4e68c1650ff24ec9091a7a021d52531e0 (in the negative mode) and http://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=bc6d018bf9d044d09353515f1ed7bca (in the positive mode). The following settings were used for generation of the network: minimum pairs cos 0.6; parent mass tolerance, 2 Da; MS/MS fragment ion tolerance, 0.5 Da; network top, 10; minimum matched peaks, 5. The molecular networking data were analyzed and visualized using Cytoscape (ver. 3.7.2).

**Results**

**Study on molecular networking of Mass spectrometry of Erdong Decoction**

All the full-MS and MS/MS spectra were obtained in high-resolution FT-MS for robust identification. In order to quickly identify the main chemical components in Erdong decoction, LC-MS/MS based molecular networking was applied. The MS data was processed through GNPS online workflow and visualized by MS/MS molecular networking. Their spectral similarities were evaluated through cosine calculation (cos θ), the larger the cos θ value,
the higher the similarity of the MS/MS fragments [7]. The results showed that the molecular networking in the negative mode was more obvious than that of the positive mode. The MS data of steroids, triterpenes, and flavones in the LC-MS/MS molecular networking of Erdong decoction were split into different groups. Herein, a total of 430 nodes was incorporated into the MS/MS molecular networking of Erdong decoction in the negative mode, rendering 30 molecular clusters and 164 unconnected nodes (Figure 2). Based on the clusters in the molecular networking, 113 compounds were rapidly identified from Erdong decoction for the first time, which including steroidal saponins, triterpenoid saponins, flavone O-glycosides and flavone C-glycosides. The typical total ion chromatograms (TIC) of Erdong decoction in the positive mode and the negative mode are presented in Figure 3. Details of the characterization of these compounds were further elaborated.

**Rapid identification of Steroidal Saponins**

Previous studies had reported that steroidal saponin was one of the main compounds of Asparagi radix [8]. Taking aspacochioside A at m/z 903.495 as an example, its MS/MS spectrum showed three characteristic fragments of m/z 757.432, m/z 595.383, and m/z 433.330, which in turn lost rhamnosyl, glucosyl and glucosyl, the fragment of m/z 433.330 corresponding to the aglycone of aspacochioside A (Figure S1). The fragmentation scheme of aspacochioside A was further elaborated in Figure S1. In comparison to aspacochioside A, its adjacent node of m/z 919.491 gave a MS/MS spectrum showing identical aglycone and three identical characteristic fragments, with different [M-H]- ion (Figure 4a). The node of m/z 919.491 was preliminarily deduced as aspacochioside A analogue with one more hydroxyl group to the rhamnose of aspacochioside A, finally annotated as 3-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl-26-O-β-D-glucopyranosyl-25S-5β-furostan-3β,22α26-triol according literature [8]. According to the clusters, the structures of these compounds could be rapidly identified. Sixteen steroidal saponins were identified from Asparagus radix and 14 steroidal saponins were identified from Anemarrhe naerhizoma by comparison with reported literatures [8-10] (Table 1), and they were annotated in red and light green in Figure 2, respectively.

Steroidal saponins in Erdong Decoction are partly from Asparagus radix and Anemarrhe naerhizoma, and partly from Ophiopogonis radix. But only two steroidal saponins from Ophiopogonis radix were identified by comparison with literature [11] (Table 1).

**Rapid identification of triterpenoid saponins**

Triterpenoid saponins in Erdong decoction were derived from Glycyrrhizae radix and Ginseng radix [12]. Glycyrrhizin acid as the mainly active compound in Glycyrrhizae radix, its MS/MS fragments mainly showed the fragment of disaccharides chain at m/z 351.057 and the weak signal of aglycone fragment at m/z 469.332. The fragmentation scheme of glycyrrhizin acid was further elaborated in Figure 5a. In comparison to glycyrrhizin acid, its adjacent node of m/z 837.392 gave a MS/MS spectra of an identical disaccharides chain fragment, with different fragment of aglycone at m/z 485.330 (Figure 4b). The node of m/z 837.392 was preliminarily deduced as glycyrrhizin acid analogue with one more hydroxyl group in the aglycone moiety of glycyrrhizin acid, finally annotated as macedonoside A by comparison with literature [12]. Based on the cluster, twenty-four steroidal saponins were identified rapidly from Glycyrrhizae radix by comparison with literatures [12,13], including 3 groups of isomers (Table 1), they were annotated in dark green in Figure 2.

Ginsenosides could not be quickly identified by LC-MS/MS molecular networking under the condition of negative mode. Only 8 triterpenoid saponins from ginseng were identified by comparison with literatures [14,15] (Table 1), they were annotated in purple in Figure 2.

**Rapid identification of Flavonoids**

The flavonoids in Erdong decoction were derived from four herbs, Anemarrhe naerhizoma, Nelumbinis folium, Glycyrrhizae radix and Scutellariae radix. According to the difference of glycoside bond atoms, flavones in Erdong decoction were divided into two types. Identified flavonoids were annotated in blue for flavone O-glycosides and light blue for flavone C-glycosides (Figure 2).

**Flavone O-glycosides**

The flavone O-glycosides in the Erdong decoction are mainly from Scutellariae radix and Glycyrrhizae radix. The types of aglycone are mainly flavone and flavanone. It was well known that baicalin and wogonoside were mainly active components in Scutellariae radix [16,17]. Peak 72 was identified as wogonoside by comparison with its standard compound, and its MS/MS spectra showed three characteristic fragments of m/z 283.061, m/z 268.038, and m/z 240.042, which in turn lost C6H10O5, CH3 and CO, the fragment of m/z 283.061 corresponding to the aglycone moiety of wogonoside by the loss of Da 176 (C6H10O5) from the [M-H]- ion [18] (Figure S2). The fragmentation scheme of wogonoside was further elaborated in Figure S2. In comparison to wogonoside, its adjacent node of m/z 475.088 gave a MS/MS spectrum of different aglycone fragment at m/z 299.056 by the loss of Da 176 (C6H10O5), with one more hydroxyl group to the aglycone of wogonoside. The node of m/z 475.088 was annotated as the isomer of hydroxyl wogonoside according to literatures [16,19] (Figure 4c). Notably, another adjacent node of m/z 445.078 was connected to wogonoside in the molecular networking with a relatively low similarity (Figure 4c). Comparing with wogonoside, the node of m/z 445.078 gave a MS/MS spectrum showing a different aglycone fragment at m/z 269.045 by the loss of Da 176 (C6H10O5), with one less methyl group to the aglycone of wogonoside. The node of m/z 445.078 was annotated as baicalin by comparison with standard compound. Basing on the cluster, forty-one flavone O-glycosides were identified from Scutellariae radix and Glycyrrhizae radix by comparison with literatures [12,16,17].

Some studies have shown that liquiritin and isoliquiritin are the active compounds in Glycyrrhizae radix [12]. It is noteworthy that some of isomers could not be distinguished by MS/MS and MN, but these isomers could be separated by retention time during LC-MS/MS analysis. Therefore, two
groups of flavone isomers (peaks 9, 11, 44, 48, 14, 38, and 46) from Glycyrrhiza radix were identified by comparison with literatures [12,13] (Table 1).

Flavone C-glycosides

The flavone C-glycosides in Erdong decoction were mainly from Scutellariae radix and Anemarrhe naerhizoma. Taking peak 19 at m/z 547.146 as an example, at m/z 487.125, m/z 457.114, m/z 427.123 involved serial losses of 60 Da, 90 Da, 120 Da, revealed that these compounds were flavone C-glycosides with two attached saccharides: glucose and arabinose [16]. So peak 19 was identified as Chrysirin 6-C-arabinoside-8-C-glucoside. The fragmentation scheme of Chrysirin 6-C-arabinoside-8-C-glucoside was further elaborated in Figure 4d and it shows special cleavage rule in the glucosyl part. In comparison to Chrysirin 6-C-arabinoside-8-C-glucoside, its adjacent node of m/z 561.161 gave a MS/MS spectrum showing two characteristic fragments at m/z 471.130 and at m/z 441.118 by the loss of 90 Da, 120 Da, and so one more methyl group should be connected to the aglycone of Chrysirin 6-C-arabinoside-8-C-glucoside. The node of m/z 561.161 was annotated as 5-hydroxy-7-methoxyflavone 6-C-arabinoside-8-C-glucoside or 7-hydroxy-5-methoxyflavone 6-C-arabinoside-8-C-glucoside [16] (Figure 4d). Basing on the cluster, six flavone C-glycosides were identified from Scutellariae radix by comparison with literatures [16,17].

Previous studies showed that the flavonoids from Anemarrhe naerhizoma were main xanthones, which was a special structure type of flavonoids, so it was not clustered with most of flavonoids in the molecular networking. Finally, 3 flavone C-glycosides were identified from Anemarrhe naerhizoma by comparison with literature [10] (Table 1).

Identification of alkaloids

A total of 169 nodes were incorporated into the MS/MS molecular network (in the positive mode) of the Erdong decoction, rendering 15 molecular clusters and 88 unconnected nodes. Besides the above three types of main compounds detected in Erdong decoction in negative mode, there are alkaloids from Nelumbinis folium mainly detected in positive mode. The mass spectrum of nuciferine at m/z 296.164 was detected and its MS/MS spectrum showed four characteristic fragments of m/z 265.123, m/z 250.098, m/z 234.103 and m/z 235.075 (Figure S3). The fragmentation scheme of nuciferine was further elaborated in Figure S3. It was well known that alkaloids were the major active compound of Nelumbinis folium [20], however, it was not shown in molecular networking and alkaloids could not be rapidly identified through the clusters in the LC-MS/MS molecular networking due to its various structural types. Finally, a total of 10 alkaloids were identified from Nelumbinis folium by comparison with literatures [20,21] (Table 2).

Discussion

According to the above results, LC-MS/MS molecular networking is suitable for the rapid identification of steroidal saponins, glycyrrhizin saponins, and flavonoids. Because of the stable structure of steroidal saponins and glycyrrhizin saponins, and special cleavage rule of flavone C-glycosides, their analogues in the LC-MS/MS molecular networking were obviously clustered with a high similarity. Based on the clusters, the structures of these compounds could be rapidly identified by MN. In addition, the flavone O-glycosides obviously clustered in LC-MS/MS molecular networking, but the similarity between nodes was low, which might be due to different substituents sites on aglycones. Therefore, the identification of flavone O-glycosides could be facilitated by the combination of LC-MS/MS and molecular networking, but standard compounds are needed for the finally identification of isomers.

Notably, MS/MS-based molecular networking technique is not suitable for the rapid identification of compounds without cluster in MN. Steroidal saponins from Ophiopogonis radix and triterpenoid saponins from Ginseng radix in Erdong decoction couldn't be rapid identified, which might be due to their low content caused by both low formula ratio in Erdong decoction and low content in each herb itself. According to the unpublished quantification data by our laboratory, the content of saponins from Glycyrrhiza Radix Et Rhizoma, Anemarrhe Naerhizoma, Asparagi Radix are very high, whereas the content of saponins from Ophiopogonis Radix and Ginseng Radix Et Rhizoma are very low. The content of those compounds might be too low to generate fragment of aglycones in this study, so the MS/MS fragments of these compounds were not clustered in this study. The second type of compounds without cluster in the molecular networking is the alkaloids from Nelumbinis folium. That might be due to the various types of structural framework of alkaloids, which leads to the MS/MS fragments of alkaloids doesn't have a certain similar.

Conclusions

In this study, the combination of LC-HRMS and molecular networking was applied to rapidly identify compounds in Erdong decoction as a case study to demonstrate the application of this technique in complex TCM formula. MS/MS-based molecular networking technique is very useful for the rapid identification of major components in CCF. Finally, 113 compounds were rapidly identified, the types of these compounds mainly include steroidal saponin, triterpenoid saponins and flavonoids in Erdong decoction. MS/MS-based molecular networking greatly improves the efficiency of chemical components identification in CCF.

Abbreviations

CCF: Chinese Classical Formula; UHPLC-LTQ-Orbitrap-MS/MS: Ultra-high pressure liquid chromatography-linear ion trap-orbitrap high resolution mass spectrometry; TCM: Traditional Chinese medicine; HRMS: High-resolution mass spectrometry; LC-MS: Liquid chromatography mass spectrometry; MN: Molecular networking.
Declarations

Competing interests
The authors declare no conflict of interest.

Authors' contribution
YD, SC, and ZH designed the experiment. RJ, XW, and SS carried out the experiment. QJ contributed analysis tools. XX contributed to the data analysis. XX, QJ, SC, and YD wrote the manuscript.

Availability of data and materials
All data included in this article are available from the corresponding author upon request.

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Tables
Table 1
Identification of the chemical constituents of Erdong decoction by UHPLC-MS in the negative mode.

| Peak no. | \( T_R \) (min) | Formula | Adduct \( \text{Mass m/z} \) | Experimental \( \text{Mass m/z} \) | Theoretical \( \text{Mass m/z} \) | Mass Error (ppm) | Fragment ions | Identification          | Source |
|----------|-----------------|---------|-----------------------------|-------------------------------|---------------------------------|-----------------|-----------------|-------------------------|-------|
| 1        | 2.21            | \( \text{C}_{25}\text{H}_{28}\text{O}_{16} \) | \([\text{M-H}]^-\)            | 583.1306                      | 583.1294                       | 2.039           | 493.0986\(\ldots\) | Neomangiferin            | A     |
| 2        | 2.54            | \( \text{C}_{21}\text{H}_{22}\text{O}_{12} \) | \([\text{M-H}]^-\)            | 465.1032                      | 465.1028                       | 0.984           | 303.0524\(\ldots\) | Spiraeoside              | S     |
| 3        | 4.13            | \( \text{C}_{19}\text{H}_{18}\text{O}_{11} \) | \([\text{M-H}]^-\)            | 421.0778                      | 421.0765                       | 2.974           | 285.0404\(\ldots\) | Mangiferin               | A     |
| 4        | 4.33            | \( \text{C}_{21}\text{H}_{21}\text{O}_{11} \) | \([\text{M-H}]^-\)            | 449.1087                      | 449.1078                       | 1.987           | 259.0246\(\ldots\) | Taxifolin 7-rhamnoside   | N     |
| 5        | 4.49            | \( \text{C}_{19}\text{H}_{18}\text{O}_{11} \) | \([\text{M-H}]^-\)            | 421.0778                      | 421.0765                       | 3.045           | 285.0404\(\ldots\) | Isomangiferin            | A     |
| 6        | 6.30            | \( \text{C}_{27}\text{H}_{32}\text{O}_{14} \) | \([\text{M-H}]^-\)            | 579.1721                      | 579.1708                       | 2.224           | 255.0662\(\ldots\) | Liquiritigenin 7,4'-di-O- | G     |
| 7        | 6.65            | \( \text{C}_{26}\text{H}_{28}\text{O}_{14} \) | \([\text{M-H}]^-\)            | 563.1406                      | 563.1395                       | 1.826           | 503.1200\(\ldots\) | Apigenin 6-C-glucoside-8-C-arabinoside | S     |
| 8        | 7.12            | \( \text{C}_{26}\text{H}_{28}\text{O}_{16} \) | \([\text{M-H}]^-\)            | 595.1307                      | 595.1294                       | 2.317           | 255.0661\(\ldots\) | Quercetin-3-O-sambubioside | N     |
| 9        | 8.01            | \( \text{C}_{21}\text{H}_{22}\text{O}_{9} \) | \([\text{M-H}]^-\)            | 417.1194                      | 417.1180                       | 3.288           | 255.0661\(\ldots\) | Neoliquiritin            | G     |
| 10       | 8.43            | \( \text{C}_{27}\text{H}_{30}\text{O}_{14} \) | \([\text{M-H}]^-\)            | 577.9597                      | 577.9599                       | -0.502          | 541.9865\(\ldots\) | 5-Hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl 2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside | G     |
| 11       | 8.46            | \( \text{C}_{27}\text{H}_{30}\text{O}_{14} \) | \([\text{M-H}]^-\)            | 517.0400                      | 517.0402                       | -0.217          | 255.0655\(\ldots\) | liquiritin               | G     |
| 12       | 8.50            | \( \text{C}_{26}\text{H}_{14}\text{O}_{12} \) | \([\text{M-H}]^-\)            | 547.1458                      | 547.1446                       | 2.180           | 255.0661\(\ldots\) | 1',3',4',5',6',8',8''-Octahydroxy-9H,9'H-2,2'-bixanthene-9,9'-dione | G     |
| 13       | 8.67            | \( \text{C}_{26}\text{H}_{28}\text{O}_{13} \) | \([\text{M-H}]^-\)            | 549.1614                      | 549.1603                       | 2.135           | 255.0661\(\ldots\) | Isomer of chrysin 6-C-arabinoside-8-C-glucoside | S     |
| 14       | 8.78            | \( \text{C}_{26}\text{H}_{30}\text{O}_{13} \) | \([\text{M-H}]^-\)            | 549.1614                      | 549.1603                       | 2.135           | 255.0661\(\ldots\) | liquiritin apioside      | G     |

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| Peakno. | $T_R$ (min) | Formula | Adduct ion | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment Ions | Identification | Source |
|--------|-------------|---------|------------|-----------------------|----------------------|------------------|---------------|---------------|--------|
| 15     | 8.86        | C$_{32}$H$_{40}$O$_{18}$ | [M+H]$^+$ | 711.2114 | 711.2131 | -2.335 | 549.1617$\pm$255.0660 | 153.0176$\pm$135.0073 | 119.0487 |
| 16     | 9.22        | C$_{21}$H$_{20}$O$_{12}$ | [M+H]$^+$ | 463.0884 | 463.0871 | 2.694 | 301.0346$\pm$300.0276 | 272.0300$\pm$271.0249 | 255.0298 | 178.9979 | 151.0024 | Hyperoside | N |
| 17     | 9.23        | C$_{22}$H$_{30}$O$_{16}$ | [M+H]$^+$ | 609.1450 | 609.1450 | 0.015 | 300.0277$\pm$271.0251 | 255.0297 | 178.9977 |
| 18     | 9.26        | C$_{21}$H$_{20}$O$_{10}$ | [M+H]$^+$ | 431.0972 | 431.0973 | -0.100 | 341.0667$\pm$311.0565 | 293.0613 | 269.0455 |
| 19     | 9.33        | C$_{26}$H$_{28}$O$_{13}$ | [M+H]$^+$ | 547.1457 | 547.1446 | 1.961 | 487.1252$\pm$457.1140 | 427.1031 | 367.0822 | 337.0718 | Chrysin 6-Carabinoside-8-C-glucoside | S |
| 20     | 9.42        | C$_{26}$H$_{30}$O$_{14}$ | [M+H]$^+$ | 565.1558 | 565.1552 | 1.023 | 438.8078$\pm$295.0642 | 271.0612 |  |
| 21     | 9.48        | C$_{21}$H$_{18}$O$_{13}$ | [M+H]$^+$ | 477.0676 | 477.0664 | 2.585 | 302.0389$\pm$301.0354 | 283.0245 | 255.0300 | 227.0338 | 178.9976 | 151.0024 | Quercetin-3-O-glucuronide | N |
| 22     | 9.59        | C$_{26}$H$_{28}$O$_{15}$ | [M+H]$^+$ | 579.1363 | 579.1344 | 3.183 | 284.0328$\pm$255.0293 | 227.0346 | 151.0025 |
| 23     | 9.68        | C$_{23}$H$_{24}$O$_{13}$ | [M+H]$^+$ | 507.1151 | 507.1133 | 3.595 | 345.0613$\pm$330.0382 | 315.0154 |
| 24     | 9.74        | C$_{21}$H$_{20}$O$_{12}$ | [M+H]$^+$ | 463.0884 | 463.0871 | 2.694 | 300.0276$\pm$271.0250 | 255.0296 | 178.9976 | 151.0024 |
| 25     | 9.86        | C$_{21}$H$_{18}$O$_{12}$ | [M+H]$^+$ | 461.0716 | 461.0715 | 0.234 | 285.0407$\pm$267.0296 | 175.0238 |
| 26     | 10.61       | C$_{26}$H$_{28}$O$_{13}$ | [M+H]$^+$ | 547.1457 | 547.1446 | 1.961 | 457.1138$\pm$427.1029 | 367.0823 | 337.0719 |  |
| 27     | 11.11       | C$_{26}$H$_{28}$O$_{13}$ | [M+H]$^+$ | 547.1458 | 547.1446 | 2.070 | 457.1140$\pm$427.1028 | 367.0822 | 337.0720 |  |
| 28     | 11.26       | C$_{27}$H$_{28}$O$_{16}$ | [M+H]$^+$ | 607.1306 | 607.1294 | 2.074 | 431.0992$\pm$269.0456 |
| 29     | 11.35       | C$_{27}$H$_{28}$O$_{16}$ | [M+H]$^+$ | 607.1303 | 607.1294 | 1.563 | 445.0771$\pm$431.0983 | 269.0455 |

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| Peakno. | T_R (min) | Formula | Adduct ion | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment Ions | Identification | Source |
|---------|-----------|---------|------------|-----------------------|---------------------|------------------|---------------|---------------|--------|
| 30      | 11.59     | C_{2}H_{20}O_{9} | [M+H]^+  | 415.1035 | 415.1024 | 2.629 | 295.0613, 267.0663, 251.0709, 223.0758 | Chrysirin 8-C-β-glucoside | S |
| 31      | 11.82     | C_{2}H_{24}O_{10} | [M+H]^+  | 459.1299 | 459.1286 | 2.781 | 255.0661, 153.0181, 135.0073, 119.0487 | 6'-Acetylisoliquiritin | G |
| 32      | 11.91     | C_{2}H_{24}O_{13} | [M+H]^+  | 507.1144 | 507.1133 | 2.037 | 344.0537, 329.0306, 316.0585 | Viscidulin III-2'-O-β-D-glucopyranoside | S |
| 33      | 12.35     | C_{2}H_{30}O_{13} | [M+H]^+  | 561.1610 | 561.1603 | 1.324 | 471.1297, 441.1179, 281.0830 | 5-Hydroxy-7-methoxyflavone 6-C-arabinoside-8-C-glucoside or 7-Hydroxy-5-methoxyflavone 6-C-arabinoside-8-C-glucoside | S |
| 34      | 12.36     | C_{2}H_{20}O_{12} | [M+H]^+  | 475.0880 | 475.0871 | 1.847 | 299.0563, 284.0327 | Isomer of hydroxyl oroxylin A 7'-O-glucuronide or hydroxyl wogonoside | S |
| 35      | 12.37     | C_{4}H_{26}O_{20} | [M+H]^+  | 935.4862 | 935.4846 | 1.699 | 773.4357, 611.3790, 449.3284 | Timosaponin E | A |
| 36      | 12.59     | C_{5}H_{34}O_{25} | [M+H]^+  | 1095.5241 | 1095.5218 | 2.059 | 933.4723, 771.4182, 404.0874 | (2α,3β,5α,6β,25R,26-Dihydroxyisoprostanes-3-yl)-β-D-glucopyranosyl-(1→2)-(β-D-glucopyranosyl-(1→3))-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosid | A |
| 37      | 12.65     | C_{3}H_{26}O_{11} | [M+H]^+  | 585.1365 | 585.1391 | -4.440 | 549.1618, 539.2637, 417.1174, 297.0774, 255.0662 | (3'-4-Hydroxy-3-methoxylphenyl)-6-[(2R,3R)-3,5,7-trihydroxy-4-oxo-3,4-dihydro-2H-chromen-2-yl]-2,3-dihydro-1,4-benzodioxin-2-yl)methyl benzoate | G |
| 38      | 12.66     | C_{2}H_{30}O_{13} | [M+H]^+  | 549.1614 | 549.1603 | 2.026 | 255.0661, 153.0180, 135.0074, 119.0487 | Isoliquiritin apioside | G |
| 39      | 12.67     | C_{5}H_{32}O_{29} | [M+H]^+  | 1227.5653 | 1227.5641 | 1.024 | 1065.5123, 933.4720, 771.4169, 447.3155 | 3-O-β-D-xylopyranosyl(1→4)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl-26-O-β-D-glucopyranosyl-(25S)-5'-furostan-22-methoxy-3β,26-diol | As |
| 40      | 12.69     | C_{4}H_{32}O_{19} | [M+H]^+  | 961.5383 | 961.5367 | 1.678 | 799.4863, 637.4363, 475.3780, 391.2874 | 20-Glc-Rf | P |
| 41      | 12.75     | C_{4}H_{32}O_{19} | [M+H]^+  | 917.4751 | 917.4741 | 1.138 | 755.4233, 593.3687, 553.3922, 364.0068, 319.1408 | Timosaponin D | A |
| 42      | 12.80     | C_{5}H_{34}O_{24} | [M+H]^+  | 1079.5287 | 1079.5269 | 1.677 | 933.4645, 917.4766, 771.4186, 609.3615 | Alliinomside B | A |
| 43      | 12.87     | C_{2}H_{32}O_{10} | [M+H]^+  | 459.1300 | 459.1286 | 3.107 | 255.0660, 153.0180, 135.0073, 119.0488 | 6'-Acetylisoliquiritin | G |

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| Peakno. | T<sub>R</sub> (min) | Formula | Adduction | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment Ions | Identification | Source |
|--------|-----------------|---------|-----------|----------------------|----------------------|------------------|---------------|--------------|--------|
| 44     | 12.91           | C<sub>21</sub>H<sub>22</sub>O<sub>9</sub> | [M-H]<sup>-</sup> | 417.1190             | 417.1180             | 2.281            | 255.0661, 153.0179, 119.0487 | isoliquiritin | G      |
| 45     | 12.93           | C<sub>21</sub>H<sub>18</sub>O<sub>11</sub> | [M-H]<sup>-</sup> | 445.0776             | 445.0765             | 2.477            | 270.0490, 269.0455, 251.0349, 241.0509, 223.0393 | Baicalin     | S      |
| 46     | 12.95           | C<sub>21</sub>H<sub>15</sub>O<sub>13</sub> | [M-H]<sup>-</sup> | 549.1620             | 549.1603             | 3.137            | 255.0659, 153.0182, 135.0073, 119.0490 | licuraside   | G      |
| 47     | 13.01           | C<sub>45</sub>H<sub>76</sub>O<sub>20</sub> | [M-H]<sup>-</sup> | 935.4857             | 935.4846             | 1.111            | 773.4354, 611.3803, 449.3252 | Timosaponin E1 | A      |
| 48     | 13.36           | C<sub>21</sub>H<sub>22</sub>O<sub>9</sub> | [M-H]<sup>-</sup> | 417.1195             | 417.1180             | 3.599            | 255.0662 | neoisoliquiritin | G      |
| 49     | 13.41           | C<sub>23</sub>H<sub>22</sub>O<sub>13</sub> | [M-H]<sup>-</sup> | 505.0992             | 505.0977             | 3.055            | 329.0667, 314.0435, 299.0198, 271.0250, 255.0291, 227.0344, 175.0237 | 5,6'-dihydroxy-6,7-dimethoxyflavone 2'-O-β-D-glucuronide | S    |
| 50     | 13.53           | C<sub>21</sub>H<sub>20</sub>O<sub>11</sub> | [M-H]<sup>-</sup> | 447.0930             | 447.0922             | 1.861            | 271.0613, 243.0660 | dihydrobaicalin | S      |
| 51     | 13.60           | C<sub>45</sub>H<sub>77</sub>O<sub>14</sub> | [M-H + HCOOH]<sup>-</sup> | 845.4905             | 845.4893             | 1.381            | 799.4837, 637.4315, 475.3803, 273.3054 | ginsenoside Rg1 | P      |
| 52     | 13.61           | C<sub>46</sub>H<sub>82</sub>O<sub>18</sub> | [M-H + HCOOH]<sup>-</sup> | 991.5499             | 991.5472             | 2.671            | 945.5428, 783.4907, 637.4326, 475.3786 | ginsenoside Re | P      |
| 53     | 13.69           | C<sub>33</sub>H<sub>56</sub>O<sub>15</sub> | [M-H]<sup>-</sup> | 695.1979             | 695.1970             | 1.199            | 549.1608, 531.1499, 255.0664, 153.0185, 135.0074, 119.0486 | Licorice-glycoside B | G      |
| 54     | 13.81           | C<sub>34</sub>H<sub>58</sub>O<sub>16</sub> | [M-H]<sup>-</sup> | 725.2089             | 725.2076             | 1.722            | 549.1630, 531.1491, 255.0660, 153.0179, 135.0072, 119.0488 | Licorice-glycoside A | G      |
| 55     | 13.92           | C<sub>21</sub>H<sub>18</sub>O<sub>11</sub> | [M-H]<sup>-</sup> | 445.0761             | 445.0765             | -0.961           | 270.0488, 269.0455, 249.0541, 241.0501, 225.0548 | Apigenin 7-O-glucuronide | S      |
| 56     | 14.12           | C<sub>50</sub>H<sub>84</sub>O<sub>23</sub> | [M-H]<sup>-</sup> | 1051.5342            | 1051.5320            | 2.107            | 919.4982, 889.4860, 757.4376, 595.3851, 433.3344 | Officinalisin-1 | As    |
| 57     | 14.20           | C<sub>45</sub>H<sub>76</sub>O<sub>19</sub> | [M-H]<sup>-</sup> | 919.4907             | 919.4897             | 1.103            | 757.4378, 595.3847, 433.3319 | 3-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl-26-O-β-D-glucopyranosyl-(25S)-5β-furostan-3β22a26-triol | As    |

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| Peakno. | $T_R$ (min) | Formula | Adduct ion | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment Ions | Identification | Source |
|--------|-------------|---------|------------|-----------------------|---------------------|------------------|---------------|---------------|--------|
| 58     | 14.24       | C$_{30}$H$_{64}$O$_{23}$ | [M+H]$^+$ | 1051.5327            | 1051.5320           | 0.709            | 919.4877; 889.4722; 757.4381; 594.6215; 418.5930 | 25-epi-officinalisin | As |
| 59     | 14.28       | C$_{43}$H$_{76}$O$_{21}$ | [M+H]$^+$ | 951.4787             | 951.4795            | -0.878           | 633.9669; 475.0884 | (2a,3b,5a,22S)-26-(β-D-Glucopyranosyl)-2,5,22-trihydroxyfurostan-3-yl 4-O-β-D-glucopyranosyl-β-D-glucopyranoside | As |
| 60     | 14.41       | C$_{43}$H$_{74}$O$_{17}$ | [M-CO$_2$-H]$^+$ | 841.4950            | 841.4944           | 0.716            | 781.4773; 637.4346; 475.3789 | ginsenoside mRg1 | P |
| 61     | 14.45       | C$_{43}$H$_{74}$O$_{20}$ | [M+H]$^+$ | 969.4695             | 969.4690            | 0.525            | 922.5041; 825.9856; 760.4490; 471.1639; 351.0573 | (3β,22β,22-(β-D-Glucopyranosylxy)-11-oxolean-12-en-3-yl 2-O-β-D-glucopyranuronosyl-β-D-glucopyranosiduronic acid | G |
| 62     | 14.46       | C$_{56}$H$_{92}$O$_{27}$ | [M+H + HCOOH]$^+$ | 1241.5817           | 1241.5797          | 1.584            | 1241.5817; 1195.5740; 1079.5382; 1033.5212; 917.4714; 755.4238; 455.1436 | Ophiopojaponin G | O |
| 63     | 14.55       | C$_{21}$H$_{18}$O$_{11}$ | [M+H]$^+$ | 445.0772             | 445.0765            | 2.409            | 270.0491; 269.0456; 251.0346; 241.0503; 225.0552; 223.0392 | Isomer of baicalin | S |
| 64     | 14.60       | C$_{43}$H$_{72}$O$_{19}$ | [M+H]$^+$ | 919.4914             | 919.4897            | 1.831            | 757.4382; 595.3838; 433.3329 | 3-O-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl-26-0-β-D-glucopyranosyl-(25R)-5β-furostan-3β,22α,26-triol | As |
| 65     | 14.61       | C$_{51}$H$_{96}$O$_{24}$ | [M+H]$^+$ | 1081.5433            | 1081.5425           | 0.740            | 919.4806; 757.4385; 595.3859 | 26-(Hexopyranosyloxy)-22-hydroxyfurostan-3-yl hexopyranosyl-(1→2)hexopyranosyl-(1→4)hexopyranoside | A |
| 66     | 14.65       | C$_{21}$H$_{18}$O$_{10}$ | [M+H]$^+$ | 429.0821             | 429.0816            | 1.018            | 253.0505; 175.0236; 113.0229 | Chrysirin-7-Oββ-D-glucuronic | S |
| 67     | 14.79       | C$_{22}$H$_{20}$O$_{11}$ | [M+H]$^+$ | 459.0937             | 459.0922            | 3.338            | 283.0614; 269.0411; 268.0370; 241.0481; 175.0235 | Oroxylin A-7-Oβ-D-glucuronide | S |
| 68     | 14.81       | C$_{51}$H$_{96}$O$_{23}$ | [M+H]$^+$ | 1063.5324            | 1063.5320           | 0.362            | 901.4807; 755.4263; 468.3537; 423.1946 | Timosaponin BII | A |
| 69     | 14.91       | C$_{22}$H$_{20}$O$_{12}$ | [M+H]$^+$ | 475.0882             | 475.0871            | 2.247            | 290.0563; 284.0327 | Isomer of hydroxywogonin glucuronide | S |
| 70     | 14.93       | C$_{56}$H$_{92}$O$_{28}$ | [M+H]$^+$ | 1211.5714            | 1211.5691           | 1.875            | 1079.5255; 917.4763; 865.0001; 755.4222 | Timosaponin C1 | A |
| 71     | 15.02       | C$_{43}$H$_{76}$O$_{19}$ | [M+H]$^+$ | 919.4921             | 919.4897            | 2.571            | 841.4293; 757.4416; 595.3847; 459.0930 | Timosaponin BII | A |

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| Peakno. | $T_R$ (min) | Formula | Adduct | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment Ions | Identification | Source |
|--------|-------------|---------|--------|-----------------------|---------------------|-----------------|---------------|---------------|--------|
| 72     | 15.59       | $C_{22}H_{20}O_{11}$ | [M+H]$^+$ | 459.0932             | 459.0922            | 2.205           | 283.0614; 269.0431; 268.0378; 240.0425; 175.0237 | Wogonoside | S |
| 73     | 15.70       | $C_{57}H_{44}O_{27}$ | [M+H]$^+$ | 1209.5912            | 1209.5899           | 1.088           | 1047.5446; 901.4795; 883.4755; 755.4213; 737.4127; 431.3182 | (2α,3β,5α,25R)-2-Hydroxyfurostan-3-yl β-D-glucopyranosyl(1→2)-4-O-(2,3,5-R,4S)-3-hydroxy-4-(hydroxymethyl)-4-methyltetrahydro-2-furanyl]-β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)]-β-D-galactopyranoside | A |
| 74     | 15.91       | $C_{57}H_{44}O_{22}$ | [M+H]$^+$ | 1047.5380            | 1047.5371           | 0.868           | 901.4722; 885.4497; 755.4229 | Protodioscin | As |
| 75     | 16.06       | $C_{57}H_{44}O_{22}$ | [M+H]$^+$ | 1047.5382            | 1047.5371           | 1.107           | 901.4749; 883.4813; 755.4178; 413.2992 | Protodioscin | As |
| 76     | 16.09       | $C_{50}H_{44}O_{25}$ | [M+H]$^+$ | 1083.5170            | 1083.5218           | -4.452          | 1047.5375; 901.4825; 802.9248; 755.4275; 487.1885 | (2α,3β,5α,22S,25R)-26-(β-D-glucopyranosyloxy)-2,5,22-trihydrofurostan-3-yl β-D-xylopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)]-β-D-galactopyranoside | A |
| 77     | 16.29       | $C_{50}H_{44}O_{22}$ | [M+H]$^+$ | 1035.5374            | 1035.5371           | 0.289           | 903.5004; 889.4836; 757.4378; 595.3881; 433.3307 | 3-O-α-L-rhamnopyranosyl(1→4)]-β-D-xylopyranosyl(1→2)]-β-D-glucopyranosyl(26-β-D-glucopyranosyl(25S)-5β-furostan-3β22α26-trio | As |
| 78     | 16.69       | $C_{51}H_{46}O_{23}$ | [M+H]$^+$ | 1065.5487            | 1065.5476           | 1.028           | 903.4990; 757.4362; 595.3870; 445.8120 | (5α22R)-26-(β-D-Glucopyranosyloxy)-22-hydroxypyranostan-3-yl 6-deoxy-α-L-mannopyranosyl(1→4)]-β-D-glucopyranoside | A |
| 79     | 16.87       | $C_{48}H_{52}O_{22}$ | [M+H]$^+$ | 999.4452             | 999.4431            | 2.041           | 837.3885; 351.0569 | 24-hydroxy-licoricesaponin A3 | G |
| 80     | 16.90       | $C_{51}H_{56}O_{23}$ | [M+H]$^+$ | 1065.5483            | 1065.5476           | 0.680           | 903.4954; 757.4395; 739.4266; 595.3826; 433.3332 | 3-O-α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl(25S)-5β-furostan-3β22α26-triol | As |
| 81     | 17.24       | $C_{48}H_{48}O_{18}$ | [M+H]$^+$ | 903.4975             | 903.4948            | 0.363           | 757.4323; 595.3828; 433.3293 | aspachioside A | As |
| 82     | 17.47       | $C_{48}H_{48}O_{18}$ | [M+H]$^+$ | 903.4968             | 903.4948            | 2.256           | 757.4388; 595.3868; 433.3327 | Isomer of aspachioside A | As |
| 83     | 17.53       | $C_{48}H_{48}O_{19}$ | [M+H]$^+$ | 895.3964             | 895.3958            | 0.619           | 456.4406; 429.6882; 351.0563 | Hydroxyacetoxyglycyrrhizin | G |
| 84     | 17.75       | $C_{48}H_{48}O_{18}$ | [M+H]$^+$ | 853.3855             | 853.3852            | 0.303           | 351.0568 | 22-Hydroxy-licoricesaponin G2 | G |
| 85     | 19.31       | $C_{48}H_{22}O_{21}$ | [M+H]$^+$ | 983.4494             | 983.4482            | 1.184           | 821.3983; 351.0575 | Licorice-saponin A3 | G |

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| Peakno. | $T_R$ (min) | Formula | Adduct ion | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment Iонаs | Identification | Source |
|--------|-------------|---------|------------|-----------------------|----------------------|------------------|----------------|---------------|--------|
| 86     | 19.61       | C$_{42}$H$_{60}$O$_{17}$ | [M+H]$^+$ | 835.3760              | 835.3747             | 1.560            | 801.4187$\rightarrow$ 443.5862$\rightarrow$ 381.5747$\rightarrow$ 351.0575 | formylglycyrrhizin acid | G |
| 87     | 19.71       | C$_{50}$H$_{62}$O$_{22}$ | [M+H]$^+$ | 1033.5227             | 1033.5214            | 1.267            | 901.4716$\rightarrow$ 739.4283$\rightarrow$ 577.3704$\rightarrow$ 427.2860 | 3-O-$\beta$D-glucopyranosyl (1$\rightarrow$4)-[$\beta$D-glucopyranosyl (1$\rightarrow$2)-[$\beta$D-glucopyranosyl (1$\rightarrow$2)]-26-O-$\beta$D-glucopyranosyl-(25S)-5$\beta$-furostane-20 (22)-ene-3$\beta$-26-diol | As |
| 88     | 19.73       | C$_{44}$H$_{64}$O$_{18}$ | [M+H]$^+$ | 879.4027              | 879.4009             | 2.068            | 351.0570$\rightarrow$ 193.0346$\rightarrow$ 175.0236$\rightarrow$ 113.0229 | 22β-Acetoxyglycyrrhizin | G |
| 89     | 19.78       | C$_{43}$H$_{74}$O$_{18}$ | [M+H]$^+$ | 901.4808              | 901.4791             | 1.873            | 739.4278$\rightarrow$ 577.3749$\rightarrow$ 356.9583 | Xilingsaponin B | A |
| 90     | 19.83       | C$_{42}$H$_{62}$O$_{17}$ | [M+H]$^+$ | 837.3911              | 837.3903             | 0.935            | 351.0570          | Gusenoside P2 | G |
| 91     | 19.89       | C$_{53}$H$_{64}$O$_{23}$ | [M+H]$^+$ | 1063.5335             | 1063.5320            | 1.396            | 901.4749$\rightarrow$ 739.4255$\rightarrow$ 577.3785$\rightarrow$ 445.3186 | 3-O-$\beta$D-glucopyranosyl (1$\rightarrow$4)-[$\beta$D-glucopyranosyl (1$\rightarrow$2)-[$\beta$D-glucopyranosyl (1$\rightarrow$2)]-26-O-$\beta$D-glucopyranosyl-(25S)-5$\beta$-furostane-20 (22)-ene-3$\beta$-26-diol | As |
| 92     | 20.01       | C$_{53}$H$_{64}$O$_{26}$ | [M-CO2-H]$^+$ | 1149.6069             | 1149.6051            | 1.566            | 1149.6069$\rightarrow$ 1107.5963$\rightarrow$ 945.5444$\rightarrow$ 783.4910$\rightarrow$ 621.4361$\rightarrow$ 459.3843 | Ginsenoside mRb1 | P |
| 93     | 20.04       | C$_{47}$H$_{60}$O$_{17}$ | [M+H$+$ HCOOH]$^+$ | 965.4387 | 965.4377 | 1.025 | 919.4950$\rightarrow$ 758.4404$\rightarrow$ 497.1143$\rightarrow$ 435.1156 | Gypenoside IX | P |
| 94     | 20.09       | C$_{48}$H$_{76}$O$_{19}$ | [M+H]$^+$ | 955.4913              | 955.4897             | 1.699            | 793.4381$\rightarrow$ 731.4389$\rightarrow$ 613.3751$\rightarrow$ 569.3850$\rightarrow$ 523.3790$\rightarrow$ 455.3533 | Ginsenoside Ro | P |
| 95     | 20.11       | C$_{59}$H$_{92}$O$_{25}$ | [M-CO2-H]$^+$ | 1119.5964             | 1119.5946            | 1.675            | 1077.5857$\rightarrow$ 945.5567$\rightarrow$ 915.5332$\rightarrow$ 783.4905$\rightarrow$ 621.4422$\rightarrow$ 459.3855 | Ginsenoside mRb2 | P |
| 96     | 20.13       | C$_{45}$H$_{74}$O$_{17}$ | [M+H]$^+$ | 885.4871              | 885.4842             | 3.199            | 739.4278$\rightarrow$ 577.3763$\rightarrow$ 484.2304 | 3-O-[$\alpha$L-rhamnopyranosyl (1$\rightarrow$4)-[$\beta$D-glucopyranosyl (1$\rightarrow$2)-[$\beta$D-glucopyranosyl (1$\rightarrow$2)]-26-O-$\beta$D-glucopyranosyl-(25S)-5$\beta$-furostane-20 (22)-ene-3$\beta$-26-diol | As |
| 97     | 20.16       | C$_{48}$H$_{60}$O$_{16}$ | [M+H]$^+$ | 819.3818              | 819.3815             | 2.085            | 351.0568$\rightarrow$ 193.0346$\rightarrow$ 175.0237$\rightarrow$ 113.0229 | licorice-saponin E2 | G |
| 98     | 20.18       | C$_{48}$H$_{60}$O$_{17}$ | [M+H]$^+$ | 859.3739              | 859.3747             | -0.904           | 837.3852$\rightarrow$ 797.3743$\rightarrow$ 351.0557 | methyllicorice-saponin Q2 | G |
| 99     | 20.19       | C$_{42}$H$_{62}$O$_{17}$ | [M+H]$^+$ | 837.3916              | 837.3903             | 1.521            | 351.0570          | Macedonoside A | G |

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| Peakno. | T<sub>R</sub> (min) | Formula | Adduct ion | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment Ions | Identification | Source |
|--------|-----------------|---------|------------|-----------------------|----------------------|-----------------|--------------|---------------|--------|
| 100    | 20.28           | C<sub>44</sub>H<sub>72</sub>O<sub>20</sub> | [M-H]     | 967.4548             | 967.4533             | 1.498           | 645.3641\(\text{m}+\text{n}\) | (3β,22β)-23-Hydroxy-29-oxo-22,29-epoxyolean-12-en-3β-yl 5-deoxy-α-L-mannopyranosyl-(1→2)-β-D-glucopyranuronosyl-(1→2)-βD-glucopyranosiduronic acid | G      |
| 101    | 20.29           | C<sub>44</sub>H<sub>72</sub>O<sub>17</sub> | [M-H]     | 863.4081             | 863.4082             | 2.575           | 351.0566\(\text{m}+\text{n}\) | 22β-acetoxyglycyrrhaldehyde | G      |
| 102    | 20.45           | C<sub>44</sub>H<sub>72</sub>O<sub>17</sub> | [M-H]     | 837.3915             | 837.3903             | 1.377           | 595.3846\(\text{m}+\text{n}\) | licorice-saponin Q2 | G      |
| 103    | 20.47           | C<sub>39</sub>H<sub>66</sub>O<sub>14</sub> | [M-H]     | 757.4377             | 757.4369             | 1.132           | 351.0568\(\text{m}+\text{n}\) | Anemarrhenasaponin I or II | A      |
| 104    | 20.50           | C<sub>42</sub>H<sub>62</sub>O<sub>16</sub> | [M-H]     | 821.3968             | 821.3954             | 1.678           | 352.0605\(\text{m}+\text{n}\) | Glycyrrhizin acid | G      |
| 105    | 20.52           | C<sub>42</sub>H<sub>62</sub>O<sub>16</sub> | [M-H]     | 823.4128             | 823.4111             | 1.778           | 721.3478\(\text{m}+\text{n}\) | licorice-saponin J2 | G      |
| 106    | 20.74           | C<sub>42</sub>H<sub>62</sub>O<sub>17</sub> | [M-H]     | 837.3918             | 837.3903             | 1.736           | 351.0573\(\text{m}+\text{n}\) | licorice-saponin G2 | G      |
| 107    | 20.75           | C<sub>39</sub>H<sub>66</sub>O<sub>14</sub> | [M-H+HCOOH] | 799.4072             | 799.4111             | 4.869           | 799.4072\(\text{m}+\text{n}\) | Ophiopojaponin Ra | O      |
| 108    | 20.79           | C<sub>42</sub>H<sub>62</sub>O<sub>15</sub> | [M-H]     | 807.4177             | 807.4161             | 1.873           | 351.0572\(\text{m}+\text{n}\) | licorice-saponin B2 | G      |
| 109    | 20.88           | C<sub>42</sub>H<sub>62</sub>O<sub>16</sub> | [M-H]     | 821.3971             | 821.3954             | 2.043           | 352.0600\(\text{m}+\text{n}\) | licorice-saponin H2 | G      |
| 110    | 20.95           | C<sub>39</sub>H<sub>66</sub>O<sub>14</sub> | [M-H]     | 755.4227             | 755.4212             | 1.982           | 593.3716\(\text{m}+\text{n}\) | Timosaponin A1 | A      |
| 111    | 21.06           | C<sub>42</sub>H<sub>62</sub>O<sub>16</sub> | [M-H]     | 821.3972             | 821.3954             | 2.201           | 352.0616\(\text{m}+\text{n}\) | licorice-saponin K2 | G      |
| 112    | 21.13           | C<sub>42</sub>H<sub>62</sub>O<sub>16</sub> | [M-H]     | 821.3970             | 821.3954             | 1.897           | 352.0634\(\text{m}+\text{n}\) | Apioglycyrrhizin | G      |
| 113    | 21.57           | C<sub>42</sub>H<sub>62</sub>O<sub>15</sub> | [M-H]     | 805.4006             | 805.4005             | 0.090           | 351.0572\(\text{m}+\text{n}\) | licorice-saponin C2 | G      |

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Table 2
Identification of the chemical constituents of Erdong decoction by UHPLC-MS in the positive mode.

| Peak no. | T_R (min) | Formula  | Adduct ion | Experimental Mass m/z | Theoretical Mass m/z | Mass Error (ppm) | Fragment ions | Identification       | Source |
|----------|-----------|----------|------------|-----------------------|----------------------|------------------|---------------|----------------------|--------|
| 114      | 6.43      | C_{19}H_{23}NO_{3} | [M + H]^+ | 314.1746             | 314.1751             | -1.433           | 283.1324, 252.1144, 189.0909, 174.0670, 145.0645, 107.0494 | Armepavine | N                   |
| 115      | 6.89      | C_{18}H_{21}NO_{3} | [M + H]^+ | 300.1591             | 300.1594             | -1.100           | 283.1324, 252.1143, 189.0909, 174.0671, 145.0647, 107.0494 | NorarMepavine | N                   |
| 116      | 9.06      | C_{18}H_{19}NO_{3} | [M + H]^+ | 282.1485             | 282.1489             | -1.366           | 251.1062, 236.0828, 219.0801, 191.0853 | OmNuciferine | N                   |
| 117      | 10.84     | C_{38}H_{44}N_{2}O_{6} | [M + H]^+ | 625.3267             | 625.3272             | -0.885           | 566.4268, 489.2368, 325.0908, 206.1174, 163.0388, 121.0649 | Dauricine | N                   |
| 118      | 12.78     | C_{17}H_{15}NO_{2} | [M + H]^+ | 266.1172             | 266.1176             | -1.485           | 249.0906, 219.0801, 191.0853 | Anonaine | N                   |
| 119      | 12.82     | C_{18}H_{19}NO_{2} | [M + H]^+ | 282.1486             | 282.1489             | -0.834           | 265.1219, 250.0984, 234.1036 | N-MethylNuciferine | N       |
| 120      | 12.96     | C_{18}H_{17}NO_{2} | [M + H]^+ | 280.1330             | 280.1332             | -0.840           | 249.0907, 219.0803, 191.0854, 149.0233 | Roemerine | N                   |
| 121      | 13.07     | C_{19}H_{21}NO_{2} | [M + H]^+ | 296.1643             | 296.1645             | -0.255           | 265.1218, 250.0984, 234.1035 | Nuciferine | N                   |
| 122      | 14.2      | C_{19}H_{21}NO_{3} | [M + H]^+ | 312.1591             | 312.1594             | -0.300           | 265.1219, 250.0986, 234.1033 | Pronuciferine | N       |
| 123      | 16.96     | C_{20}H_{21}NO_{4} | [M + H]^+ | 340.1539             | 340.1543             | -1.278           | 269.1166, 233.1045, 215.0938, 197.0836, 179.0864 | Tetrahydroberberine THB | N       |

N: Nelumbinis folium.

Figures
Figure 1

A general workflow for a strategy identifying compounds rapidly of Erdong decoction.

Figure 2

MS/MS molecular networking of Erdong decoction.
Figure 3

TIC of Erdong decoction in the negative mode (a) and positive mode (b).
Figure 4

MS/MS spectra of (a) steroidal saponins, (b) triterpenoid saponins, (c) flavone O-glycosides and (d) flavone C-glycosides.
Figure 5

The proposed fragmentation pathways for (a) glycyrrhizin acid and (b) Chrys 6-C-arabinoside-8-C-glucoside in negative mode.

Supplementary Files

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