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# A comparative chemical composition and main component content of various parts of *Gentiana macrophylla* Pall. by UPLC-Q-orbitrap MS and HPLC†

 Qier Mu, Yanqiu Bai, Junni Qi and Chula Sa \*

In traditional Chinese and Mongolian medicine, the roots and flowers of *Gentiana macrophylla* Pall. (*G. macrophylla*) were used as medicinal parts. Yet, no systematic study on the chemical composition and content of the various medicinal parts of *G. macrophylla* has been reported. Therefore, the aim of this study was to evaluate the different medicinal parts of *G. macrophylla*, through to discuss the its chemical structure and content. Liquid chromatography coupled to electrostatic orbitrap high-resolution mass spectrometry (UPLC-Q-orbitrap-MS) combined with characteristic chromatogram, and high performance liquid chromatography (HPLC) to analyze the chemical composition and content of the various medicinal parts of *G. macrophylla*, and to compare them. A total of 50 compounds were identified from *G. macrophylla*, and identify 7 characteristic components were screened, including iridoids (loganic acid, swertiamarin, gentiopicroside, and sweroside), flavonoids (isorientin and vitexin), and triterpenoids (roburic acid). The contents of iridoids and triterpenoids were higher in roots, and the contents of flavonoids were higher in flowers, stems and leaves. Thus, we can proved that iridoids and triterpenoids represented by loganic acid, swertiamarin, gentiopicroside, sweroside, and roburic acid are important quality markers of *G. macrophylla* roots. Also, the flavonoids represented by vitexin and isoorientin can be used as important quality markers for the flowers, stems, and leaves of *G. macrophylla*. And after a comprehensive analysis, we believe that *G. macrophylla*, as a medicinal plant, its roots, flowers, leaves and stems can be parts with medicinal value, which can be further developed and researched.

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## 1. Introduction

*G. macrophylla* (Fig. 1), whose genus and family belong to the Gentianaceae and *G. macrophylla* resources were primarily distributed in the Loess Plateau and the eastern Qinghai-Tibet Plateau in China. Within this region, provinces such as Shaanxi, Gansu, and Sichuan were major production areas for *G. macrophylla*, with Shaanxi being recognized as the genuine producing area for this species.<sup>1</sup> *G. macrophylla*, as a traditional medicine, has a long history and was used in different ethnic medicines. For a long time, it has been chosen to use its dried roots as medicinal parts in traditional Chinese medicine, which has the effect of dispelling wind and dehumidification, clearing heat of deficiency and dampness, that was first recorded in Shen Nong's Herbal Classic (Han Dynasty, Shen Nong Ben Cao Jing). The classic text emphasized the therapeutic advantages of

*G. macrophylla*, which are known for their anti-inflammatory, anti-rheumatic, antiviral properties. Additionally, it is recognized for its ability to promote blood circulation and reduce swelling and pain.<sup>2</sup> As a Mongolian medicine, the dried flowers of *G. macrophylla* were used as medicinal parts, which were first recorded in the Classic Canon of Mongolian Materia Medica (19th century, Meng Yao Zheng Dian), with the effect of removing "Xieriwusu" ("Xieriwusu" means rheumatism), clear heat and reduce swelling.<sup>3</sup> In various ethnomedicine, various parts of *G. macrophylla* have different medicinal values, and in particular the roots and flowers have different therapeutic effects, which may be related to the different the structure and activities of the chemical constituents contained therein. Thus, there is a pressing need for research on this issue.

According to the 2020 edition of the Pharmacopoeia of the People's Republic of China, *G. macrophylla* refers to the dried roots of various *Gentiana* genus in the Gentianaceae family, including *G. macrophylla*, *Gentiana straminea* Maxim. (*G. straminea*), *Gentiana crassicaulis* Duthie ex Burk. (*G. crassicaulis*), and *Gentiana dahurica* Fisch. (*G. dahurica*).<sup>4</sup> Only the root is listed in the Chinese Pharmacopoeia, and none of the other

College of Mongolian Medicine, Inner Mongolian Medical University, Jinshan Economic and Technological Development Zone, Hohhot 010110, China. E-mail: sachula1984@163.com

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Fig. 1 *Gentiana macrophylla* Pall. and its various parts (A: the whole plant; B: flower; C: leaf; D: stem; E: root).

parts are listed. Furthermore, from the studies conducted in recent years, no systematic and comprehensive studies have been conducted on other parts of *G. macrophylla* other than its roots.

For this reason, we selected *G. macrophylla* as our research object and initially employed the UPLC-Q-orbitrap-MS method to analyze the chemical composition of its various parts, such as root, flower, stem, leaf, and the differential components were screened by variable influence on projection (VIP) score. Subsequently, we have established comprehensive and systematic characteristic chromatogram for various parts of *G. macrophylla* by HPLC. On the basis of the completed characteristic chromatogram, we applied chemical pattern recognition methods, including principal component analysis (PCA), partial least squares discrimination analysis (PLS-DA), and other statistical techniques to analyze the multi-indicator variables from the HPLC data of characteristic chromatogram. On this basis, we used HPLC to determine the content of the indicative components in each of its parts. This method enabled us to comprehensively explore the differences in the main characteristic components of different parts of *G. macrophylla*. Finally, all the data will be synthesized to predict the quality markers of *G. macrophylla*, because quality markers are not only an important part of the quality control of traditional Chinese medicine, but also an important bridge between traditional theories and modern scientific methods. Thus, this research holds great significance for the future refinement of quality markers and the holistic development and utilization of these resources.

## 2. Materials and methods

### 2.1 Reagents and materials

The National Institutes for Food and Drug Control (Beijing, China) provided the reference standards, which included loganic acid (111865-202005, purity  $\geq 98.5\%$ ), swertiamarin (110785-202205, purity  $\geq 98.5\%$ ), gentiopicroside (110770-202219, purity  $\geq 98.1\%$ ), isoorientin (111974-201401, purity  $\geq 98.0\%$ ), vitexin (111687-202306, purity  $\geq 99.5\%$ ), oleanolic acid (110709-202109, purity  $\geq 95.8\%$ ), apigenin (111901-202004, purity  $\geq 99.4\%$ ), quercetin (100081-201610, purity  $\geq 99.1\%$ ),  $\beta$ -sitosterol (110851-201909, purity  $\geq 92.7\%$ ) and ferulic acid (110773-202316, purity  $\geq 99.3\%$ ). Foshan Bono Biotechnology Co., Ltd (Guangdong, China) provided the roburic acid (BN821-01-01, purity  $\geq 98.0\%$ ) and daucosterol (BN824-01-01, purity: qualitative only). Shanghai Hongyong Biotechnology Co., Ltd (Shanghai, China) provided the sweroside (260014-202108, purity  $\geq 98.0\%$ ). Wuhan Tianzhi Biotechnology Co., Ltd (Wuhan, China) provided the gentianine (CFS202401, purity  $\geq 98.0\%$ ) and kaempferol (CFS202401, purity  $\geq 98.0\%$ ). Thermo Fisher (Waltham, Massachusetts, USA) provided the methanol, acetonitrile, and phosphoric acid of HPLC grade. All of the aqueous solutions were made using Master-S15UVF (Shanghai, China) purified water.

*G. macrophylla* was collected from Shaanxi Province (longitude: 106.7039316, latitude: 34.6690133). All samples were identified according to plant morphology by Professor Menbayar Tu (Teaching and Research Department of Mongolian Medicine, School of Mongolian Medicine, Inner Mongolia Medical University).



## 2.2 Powder preparation

The collected whole *G. macrophylla* was laid flat on clean linen paper, ventilated at room temperature, and air dried. The completely air-dried *G. macrophylla* was sorted into four parts, including flowers, leaves, stems, and roots, crushed, sieved through a 50-mesh sieve to a fine powder, and set aside.

## 2.3 Extracts preparation

The dried flowers, leaves, stems, and roots were crushed and passed through a 50-mesh sieve. 10 g were weighed, and a 10-fold volume of 70% ethanol was added, refluxed, and extracted for 2 hours. After freeze-drying, the yields were 23.20%, 19.11%, 19.29% and 32.65%, respectively.

## 2.4 A comparative chemical composition of various parts of *G. macrophylla* by UPLC-Q-orbitrap MS

**2.4.1 Mixed standards preparation.** Pipette 1 mL of each standard solution of gentianine (0.33 mg mL<sup>-1</sup>), kaempferol (0.642 mg mL<sup>-1</sup>), daucosterol (0.34 mg mL<sup>-1</sup>), roburic acid (0.345 mg mL<sup>-1</sup>), vitexin (0.84 mg mL<sup>-1</sup>), oleanolic acid (0.312 mg mL<sup>-1</sup>), quercetin (0.477 mg mL<sup>-1</sup>), β-sitosterol (0.321 mg mL<sup>-1</sup>), ferulic acid (0.564 mg mL<sup>-1</sup>), gentiopicroside (0.38 mg mL<sup>-1</sup>), loganic acid (0.336 mg mL<sup>-1</sup>), isoorientin (0.846 mg mL<sup>-1</sup>), sweroside (0.731 mg mL<sup>-1</sup>), swertiamarin (0.782 mg mL<sup>-1</sup>), and apigenin (0.724 mg mL<sup>-1</sup>) were placed in a 25 mL volumetric flask, and methanol was added to the scale to obtain a mixed standard solution.

**2.4.2 Sample preparation.** An appropriate amount of sample of extract was weighed, 1 mL of 80% methanol was added, sonicated for 10 minutes, and centrifuged at 14 000 rpm for 10 minutes. 0.8 mL of the supernatant was transferred to a centrifuge tube, centrifuged again, and the supernatant was transferred to an injection vial pending UPLC-MS/MS analysis.

**2.4.3 UPLC-Q-orbitrap MS conditions.** LC analysis was performed on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) (Waters, MA, USA) with an Ultimate 3000 system at 35 °C using acetonitrile containing 0.1% formic acid (A) and deionised water containing 0.1% formic acid (B) as the mobile phase at a flow rate of 0.3 mL min<sup>-1</sup>. The gradient elution conditions were as follows: 100% B (0–5 min), 100–90% B (5–10 min), 90–70% B (10–25 min), 70–60% B (25–35 min), 60–50% B (35–45 min), 50–30% B (45–60 min), 30–0% B (60–80 min), 0% B (80–90 min), 0–100% B (90–91 min), and 100% B (91–100 min). Q exactive orbitrap high resolution MS analysis (qualitative analysis) was used to collect mass spectra data. The detection mode was full MS-ddMS<sup>2</sup> and the positive ion and negative ion modes were scanned simultaneously. The *m/z* range was 100–1200, the resolution of MS<sup>1</sup> was set to 70 000, and the resolution of MS<sup>2</sup> was set to 17500. The ion source voltage was 3.2 kV, the capillary temperature was 320 °C, the aux gas heater temperature was 350 °C, the sheath gas flow rate was 40 L min<sup>-1</sup>, and the aux gas flow rate was 40 L min<sup>-1</sup>. The aux gas flow rate was 15 L min<sup>-1</sup>, the AGC target was set to 1e6, TopN was set to 5 and the collision energy to trigger the MS<sup>2</sup> scan was set to 30, 40 and 50 using the stepped fragmentation voltage NCE.

**2.4.4 Data analysis.** Compound Discover 3.3 software was used to extract characteristic peaks from raw mass spectrometry data, and the mass deviation of characteristic peak element matching, molecular formula prediction, and isotope distribution matching was set within 5 ppm. The characteristic peaks were identified by the mz Cloud online database and the local, self-built mz Vault traditional Chinese medicine natural products database. The screening criteria for positive results were quality deviation <5 ppm, consistent with isotope distribution, and matching score >80 points in the mzVault best match database. The results include the Chinese and English name of the compound, molecular formula, structural formula, and peak area. Finally, the structure of the compound was determined by comparison with secondary mass spectra, fragment information, and peak areas in the database, combined with existing literature reports. Finally, the differential components were screened by VIP score.

## 2.5 A comparative characteristic chromatogram of various parts of *G. macrophylla* by HPLC

**2.5.1 Mixed standards preparation.** Pipette 1 mL of each standard solution of kaempferol (0.642 mg mL<sup>-1</sup>), vitexin (0.84 mg mL<sup>-1</sup>), quercetin (0.477 mg mL<sup>-1</sup>), gentiopicroside (0.38 mg mL<sup>-1</sup>), loganic acid (0.336 mg mL<sup>-1</sup>), isoorientin (0.846 mg mL<sup>-1</sup>), sweroside (0.731 mg mL<sup>-1</sup>), swertiamarin (0.782 mg mL<sup>-1</sup>), and apigenin (0.724 mg mL<sup>-1</sup>) were placed in a 25 mL volumetric flask, and methanol was added to the scale to obtain a mixed standard solution.

**2.5.2 Sample preparation.** 40 batches of extracts from flower (S1–S10), leaf (S11–S20), stem (S21–S30), and root (S31–S40) of *G. macrophylla* from the same source were taken and placed in a 10 mL volumetric bottle, followed by methanol under the scale, ultrasonic treatment for 15 minutes, cooling, methanol added to the scale, filtration, and analysis by HPLC.

**2.5.3 HPLC conditions.** An Agilent 1260 HPLC system with a variable wavelength detector, thermostatically controlled column compartment, autosampler, and quaternary pump (Agilent Technologies, USA) was used for the HPLC study. A ZORBAX Eclipse XDB-C<sub>18</sub> column (4.6 × 250 mm, 5 μm, Agilent Technologies, USA) was used to separate the compounds at 30 °C. Using a gradient program of 8–13% (B) at 0–7 min, 13–14% (B) at 7–13 min, 14–16% (B) at 13–18 min, 16–17% (B) at 18–22 min, 17–22% (B) at 22–25 min, 22–27% (B) at 25–30 min, 27–32% (B) at 30–35 min, and 32–42% (B) at 35–45 min, the mobile phase consisted of 0.1% aqueous phosphoric acid (A) and acetonitrile (B). 1.0 mL min<sup>-1</sup> was the flow rate. 240 nm was the detection wavelength.

**2.5.4 Establishment of characteristic chromatogram and similarity evaluation.** Chromatograms were recorded after the prepared root, flower, leaf, and stem sample solutions were obtained and analyzed in accordance with chromatographic conditions. The Similarity Evaluation System of Chromatographic Fingerprint of Traditional Chinese Medicine (2012 edition) issued by the National Pharmacopoeia Commission was used for analysis. The characteristic peak was identified by comparison with the reference substance. The peak with better



peak type and larger peak area was used as the reference peak (S) to evaluate the similarity of roots, flowers, leaves, and stems from different batches.

**2.5.5 Data analysis process.** After obtaining the HPLC data, we applied chemical pattern recognition methods, including principal component analysis (PCA), partial least squares discrimination analysis (PLS-DA), and other statistical techniques to analyze the multi-indicator variables from the HPLC data.

## 2.6 A comparative main component content of various parts of *G. macrophylla* by HPLC

### 2.6.1 A comparative main component content of gentiopicroside, loganic acid, sweroside, swertiamarin vitexin, and isoorientin of various parts of *G. macrophylla* by HPLC

**2.6.1.1 Mixed standards preparation.** Pipette 1 mL of each standard solution of vitexin ( $0.84 \text{ mg mL}^{-1}$ ), gentiopicroside ( $0.38 \text{ mg mL}^{-1}$ ), loganic acid ( $0.336 \text{ mg mL}^{-1}$ ), isoorientin ( $0.846 \text{ mg mL}^{-1}$ ), sweroside ( $0.731 \text{ mg mL}^{-1}$ ), and swertiamarin ( $0.782 \text{ mg mL}^{-1}$ ) were placed in a 25 mL volumetric flask, and methanol was added to the scale to obtain a mixed standard solution.

#### 2.6.1.2 Sample preparation

**2.6.1.2.1 Sample preparation of powder.** Accurately weigh about the appropriate amount of root, flower, stem, and leaf of *G. macrophylla* powder, placed in a 10 mL brown volumetric flask, add the appropriate amount of methanol (under the scale), ultrasonic treatment for 30 minutes, cooling, and then methanol to the volume on the scale, shaking, filtration, and take the filtrate for the test solution.

**2.6.1.2.2 Sample preparation of extract.** Accurately weigh about the appropriate amount of root, flower, stem, and leaf of *G. macrophylla* extract, placed in a 10 mL brown volumetric flask, add the appropriate amount of methanol (under the scale), ultrasonic treatment for 30 minutes, cooling, and then methanol to the volume on the scale, shaking, filtration, and take the filtrate for the test solution.

**2.6.1.3 HPLC conditions.** An Agilent 1260 HPLC system with a variable wavelength detector, thermostatically controlled column compartment, autosampler, and quaternary pump (Agilent Technologies, USA) was used for the HPLC study. A ZORBAX Eclipse XDB-C<sub>18</sub> column ( $4.6 \times 250 \text{ mm}$ ,  $5 \mu\text{m}$ , Agilent Technologies, USA) was used to separate the compounds at  $30^\circ \text{C}$ . Using a gradient program of 8–10% (B) at 0–8 min, 10–11% (B) at 8–15 min, 11–13% (B) at 15–18 min and 13–18% (B) at 18–25 min, the mobile phase consisted of 0.1% aqueous phosphoric acid (A) and acetonitrile (B).  $1.0 \text{ mL min}^{-1}$  was the flow rate. 240 nm was the detection wavelength.

**2.6.1.4 HPLC method validation.** The control, test, and blank control solutions were injected, the specificity of the HPLC analysis method was tested, and the chromatogram was recorded. An appropriate volume of the reference solution was diluted twice with methanol, serially diluted five times, and the peak area was recorded to obtain the linear range. The standard solution was injected six times in duplicate to assess precision. An appropriate amount of sample solution was taken and

stored at room temperature for 0, 4, 8, 12, 16, 20, and 24 h to determine stability. The RSD for precision and stability was determined using the relative standard deviation and mean of the relative peak areas. Six portions of *G. macrophylla* flower extract with known content were weighed, and an appropriate amount of 1:1 control was added, assayed, peak area was recorded, and recovery was evaluated. Recovery (%) =  $(m_1 - m_2)/m_3 \times 100\%$ . Where  $m_1$ ,  $m_2$ , and  $m_3$  were the measured amount, the sample content, and the amount of standard added, respectively. The RSD was determined by using the relative standard deviation box-mean of the relative recoveries. To assess the content, three batches of *G. macrophylla* flour and extract from various parts were accurately weighed and the sample solution prepared for determination.

**2.6.1.5 Data analysis process.** Following the acquisition of the HPLC data, the many indicator variables within the data were analyzed using chemical pattern recognition techniques, including statistical methods like error bars and horizontal stack bars.

### 2.6.2 A comparative main component content of roburic acid of root of *G. macrophylla* by HPLC

**2.6.2.1 Standard preparation.** Appropriate amounts of reference materials of roburic acid was placed in a 10 mL volumetric flask with methanol below the scale, sonicated for 15 minutes, cooled, and added methanol to the scale. The standard solutions with concentrations of  $0.345 \text{ mg mL}^{-1}$  was prepared and stored at  $4^\circ \text{C}$  for later use.

#### 2.6.2.2 Sample preparation

**2.6.2.2.1 Sample preparation of powder.** Accurately weigh about the appropriate amount of root of *G. macrophylla* powder, placed in a 10 mL brown volumetric flask, add the appropriate amount of methanol (under the scale), ultrasonic treatment for 30 minutes, cooling, and then methanol to the volume on the scale, shaking, filtration, and take the filtrate for the test solution.

**2.6.2.2.2 Sample preparation of extract.** Accurately weigh about the appropriate amount of root of *G. macrophylla* extract, placed in a 10 mL brown volumetric flask, add the appropriate amount of methanol (under the scale), ultrasonic treatment for 30 minutes, cooling, and then methanol to the volume on the scale, shaking, filtration, and take the filtrate for the test solution.

**2.6.2.3 HPLC condition.** The mobile phase consisted of 0.1% aqueous phosphoric acid (A) and acetonitrile (B) using a gradient program of 100% (B) in 0–33 min, and 100–55% (B) in 33–55 min. The flow rate was  $1.0 \text{ mL min}^{-1}$ . The detection wavelength was 205 nm.

**2.6.2.4 HPLC method validation.** The control, test, and blank control solutions were injected, the specificity of the HPLC analysis method was tested, and the chromatogram was recorded. An appropriate volume of the reference solution was diluted twice with methanol, serially diluted five times, and the peak area was recorded to obtain the linear range. The standard solution was injected six times in duplicate to assess precision. An appropriate amount of sample solution was taken and stored at room temperature for 0, 4, 8, 12, 16, 20, and 24 h to



determine stability. The RSD for precision and stability was determined using the relative standard deviation and mean of the relative peak areas. Six portions of *G. macrophylla* flower extract with known content were weighed, and an appropriate amount of 1:1 control was added, assayed, peak area was recorded, and recovery was evaluated. Recovery (%) =  $(m_1 - m_2)/m_3 \times 100\%$ . Where  $m_1$ ,  $m_2$ , and  $m_3$  were the measured amount, the sample content, and the amount of standard added, respectively. The RSD was determined by using the relative standard deviation box-mean of the relative recoveries. To assess the content, three batches of *G. macrophylla* flour and extract from various parts were accurately weighed and the sample solution prepared for determination. The content was calculated by the internal standard method.

**2.6.2.5 Data analysis process.** Following the acquisition of the HPLC data, the many indicator variables within the data were analyzed using chemical pattern recognition techniques, including statistical methods like error bars and horizontal stack bars.

## 3. Results and discussion

### 3.1 A comparative chemical composition of various parts of *G. macrophylla* by UPLC-Q-orbitrap MS

**3.1.1 Identification of chemical composition by UPLC-Q-orbitrap MS analysis.** Initially, the sample was injected under the conditions specified for UPLC-Q-orbitrap MS, and the characteristic peaks of the raw mass spectrometry data were extracted using Compound Discoverer 3.2 software. The mass deviation of characteristic peak element matching, molecular formula prediction and isotope distribution matching was set within 5 ppm. Online database (including mzVault, mzCloud) were used to identify the characteristic peaks. Compounds with a mass deviation of less than 5 ppm, consistent with isotope distribution and matching score greater than 80 in the online databases were selected. The compounds were matched, and the secondary fragmentation fragment ion information analysis was conducted to further accurately identify their chemical composition. The results showed that a total of 50 compounds were identified from the alcohol extracts of four parts (flower, leaf, stem and root) of *G. macrophylla* in the positive and negative ion modes (Table 1). The total ion current diagram (TIC) were shown in the Fig. 2. Out of these, among these, 21 compounds were detected in the positive ion mode, while 29 compounds were detected in the negative ion mode. This suggests that most compounds exhibit higher responses in the negative ion mode compared to the positive ion mode. The detected compounds were mainly flavonoids, iridoids, triterpenoids, organic acids, amino acids and alkaloids. By comparing the detected compounds with literature, the fragmentation rule of the following characteristic components are listed.

**3.1.1.1 Identification of flavonoids.** The mass spectrum characteristics of flavonoids were more significant, and most of them occur glycosidic bond breaking reaction, which will produce CO, H<sub>20</sub>, C<sub>2</sub>H<sub>2</sub>O and other neutral fragments after energy collision.<sup>29</sup> Take Peak 41 for example, in the negative ion

mode, the primary mass spectrum gave a quasimolecular ion peak of  $m/z$  269.04548 [M-H]<sup>-</sup>, and the predicted molecular formula was C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>. In the secondary mass spectrum, fragment ions  $m/z$  151.00360 and 117.03442 were formed by the cleavage of the C-ring of the parent ion to form a series of high-abundance and characteristic fragment ions. Combined with database search and literature reports,<sup>36</sup> it can be inferred that the compound is apigenin (additional file 1: Fig. S1†).

**3.1.1.2 Identification of terpenoids.** Iridoid terpenoids and triterpenoids were the main terpenoids in *G. macrophylla*. Iridoid compounds were glycosides formed by the combination of monoterpenes and sugars. The glycosidic bonds of iridoid compounds were very easy to break, resulting in the loss of 1 molecule or several molecules of sugar fragment ions. Since iridoid glycosides contain hydroxyl (-OH) and carboxyl (-COOH) groups, iridoid glycosides are also easy to lose H<sub>20</sub>, CO<sub>2</sub> and other fragments. Take Peak 14 as an example, in the negative ion mode, the  $m/z$  of the excimer ion was 375.12947 [M-H]<sup>-</sup>, the molecular formula was C<sub>16</sub>H<sub>24</sub>O<sub>10</sub>, and the molecular ion peak of the secondary mass spectrum removed 1 molecule C<sub>6</sub>H<sub>10</sub>O<sub>5</sub> to obtain a fragment with  $m/z$  of 213.07678. Further loss of 1 molecule CO<sub>2</sub> resulted in the formation of fragment  $m/z$  169.08688, and further removal of H<sub>20</sub> resulted in the formation of fragment  $m/z$  151.07622. According to the relevant literature,<sup>37</sup> the compound 14 was identified as loganic acid after comparison with the reference substance (additional file 2: Fig. S2†).

In the structure of triterpenoids, the structure of the six-membered rings was not easy to be broken. The six-membered rings exist in plants as free, glycosidic and ester forms, and are prone to fracture under the high-energy impact of mass spectrometry. In the positive ion mode, the excimer ion peak  $m/z$  of peak 48 was 457.36769 [M + H]<sup>+</sup>, and the molecular formula was C<sub>30</sub>H<sub>48</sub>O<sub>3</sub>. The excimer ion peak removed 1 molecule H<sub>20</sub> to obtain a fragment ion with  $m/z$  of 439.35800. According to the relevant literature,<sup>37</sup> the mass spectrum information and retention time of this compound were compared with the reference material, and compound 48 was confirmed to be oleanolic acid (additional file 3: Fig. S3†).

**3.1.1.3 Identification of organic acid compounds.** The decomposition of organic acid compounds usually loses H<sub>2</sub>O, CO, and CO<sub>2</sub> and produces fragmented ion peaks. The quasimolecular ion peak of peak 18 was  $m/z$  179.03488 [M-H]<sup>-</sup>, and its molecular formula was speculated to be C<sub>9</sub>H<sub>8</sub>O<sub>4</sub>. Its secondary fragment ions were  $m/z$  135.04531 [M-H-CO<sub>2</sub>]<sup>-</sup>, 107.05022 [M-H-CO<sub>2</sub>-CO]<sup>-</sup>. Combined with the mass spectrum cleavage law and the reports in literature,<sup>38</sup> it was presumed to be caffeic acid, its secondary mass spectrum (additional file 4: Fig. S4†).

**3.1.1.4 Identification of amino acid compounds.** The loss of fragment ions such as NH<sub>3</sub>, COOH or HCOOH was the general pattern of mass spectroscopic texture of amino acid compounds. Taking peak 1 as an example, the quasimolecular ion peak in the positive ion mode was  $m/z$  175.11917 [M + H]<sup>+</sup>, and the fragment ion  $m/z$  158.09270 [M + H-NH<sub>3</sub>]<sup>+</sup> was obtained by losing one molecule of NH<sub>3</sub>. On the other hand, lose one molecule of COOH to get fragment ion  $m/z$  130.09778 [M + H-COOH]<sup>+</sup>, or lose CH<sub>3</sub>N<sub>3</sub> to obtain  $m/z$  116.07033 [M + H-



Table 1 The compounds identified by UPLC-Q-orbitrap MS

No	RT [min]	Reference ion $m/z$	Diff. (ppm)	Formula	Fragmentations ( $m/z$ )	Identification	Classification	Flower	Leaf	Stem	Root	References
1	1.538	$[M + H]^+$	1.24	$C_6H_{14}N_4O_2$	158.09270, 130.09778, 116.07033	D-Arginine	Amino acids	✓	✓	✓	✓	5
2	1.54	$[M + H]^+$	1.35	$C_6H_9N_3O_2$	110.07152, 83.06058	L-Histidine	Amino acids	✓	✓	✓	✓	6
3	1.556	$[M + H]^+$	0.72	$C_5H_9NO_4$	102.05521, 84.04464	L-Glutamic acid	Amino acids	✓	✓	✓	✓	7
4	1.631	$[M - H]^-$	-0.88	$C_4H_6O_5$	133.01413, 115.00352	L-(-)-Malic acid	Organic acids	✓	✓	✓	✓	8
5	1.676	$[M - H]^-$	-1.13	$C_6H_6O_6$	111.00864	trans-Aconitic acid	Organic acids	✓	✓	✓	✓	9
6	1.698	$[M - H]^-$	-0.06	$C_6H_8O_7$	173.00922, 111.00866, 87.00861	Citric acid	Organic acids	✓	✓	✓	✓	10
7	1.717	$[M + H]^+$	0.82	$C_5H_{11}NO_2$	72.08106, 55.05478	Valine	Amino acids	✓	✓	✓	✓	11
8	1.818	$[M + H]^+$	0.74	$C_3H_7NO_3$	84.04462	L-Pyrogutamic acid	Amino acids	✓	✓	✓	✓	12
9	4.708	$[M + H]^+$	1.08	$C_6H_{13}NO_2$	86.0996	Isoleucine	Amino acids	✓	✓	✓	✓	12
10	5.604	$[M + H]^+$	1.27	$C_9H_{11}NO_3$	136.07591, 119.04932, 91.05442	L-Tyrosine	Amino acids	✓	✓	✓	✓	13
11	11.294	$[M + H]^+$	1.09	$C_9H_{11}NO_2$	120.08099, 103.05442, 93.07014	L-Phenylalanine	Amino acids	✓	✓	✓	✓	13
12	11.298	$[M - H]^-$	-0.99	$C_3H_6O_4$	132.03813	Glutaric acid	Organic acids	✓	✓	✓	✓	14
13	14.363	$[M + H]^+$	0.36	$C_{11}H_{12}N_2O_2$	188.07085	Tryptophan	Amino acids	✓	✓	✓	✓	12
14	14.551	$[M - H]^-$	-0.33	$C_{16}H_{24}O_{10}$	213.07678, 169.08688, 151.07622	Loganic acid	Iridoids	✓	✓	✓	✓	15
15	14.772	$[M - H]^-$	-1.03	$C_7H_6O_3$	137.02432, 93.03442	Salicylic acid	Organic acids	✓	✓	✓	✓	16
16	16.057	$[M + H]^+$	0.19	$C_{16}H_{22}O_{10}$	195.06552, 177.05492, 139.07564	Swertiamarin	Iridoids	✓	✓	✓	✓	17
17	16.312	$[M - H]^-$	-0.47	$C_7H_6O_4$	109.02933, 153.01926, 108.02158, 81.03451, 91.01901	2,3-Dihydroxybenzoic acid	Organic acids	✓	✓	✓	✓	10
18	16.93	$[M - H]^-$	-0.56	$C_9H_8O_4$	135.04531, 107.05022	Caffeic acid	Organic acids	✓	✓	✓	✓	18
19	17.129	$[M + FA - H]^-$	-0.2	$C_{16}H_{20}O_9$	121.06472	Gentiopicroside	Iridoids	✓	✓	✓	✓	19
20	17.473	$[M + FA - H]^-$	-0.16	$C_{16}H_{22}O_9$	197.08116, 179.07082, 127.03920	Sweetoside	Iridoids	✓	✓	✓	✓	20
21	17.743	$[M - H]^-$	0.25	$C_{15}H_{14}O_6$	271.06198, 245.08179, 205.05048	Catechin	Flavonoids	✓	✓	✓	✓	12
22	19.728	$[M - H]^-$	-0.88	$C_9H_8O_3$	163.04002	2-Hydroxycinnamic acid	Organic acids	✓	✓	✓	✓	21
23	19.747	$[M + H]^+$	0.11	$C_{21}H_{20}O_{11}$	81.03376, 85.02859	Isoorientin	Flavonoids	✓	✓	✓	✓	22
24	20.035	$[M - H]^-$	-0.62	$C_{11}H_{13}NO_3$	206.08220, 164.07159, 147.04510, 91.05531	N-Acetyl-L-phenylalanine	Amino acids	✓	✓	✓	✓	10
25	20.104	$[M + H]^+$	0.44	$C_{15}H_{10}O_8$	319.04520, 301.03491, 179.03438, 137.02351	Myricetin	Flavonoids	✓	✓	✓	✓	23
26	21.022	$[M - H]^-$	0.25	$C_{10}H_{10}O_4$	177.05499	Ferulic acid	Organic acids	✓	✓	✓	✓	12
27	21.255	$[M + H]^+$	-0.1	$C_{15}H_{12}O_4$	147.04419, 137.02351, 119.04929	Liquiritigenin	Flavonoids	✓	✓	✓	✓	24
28	21.392	$[M - H]^-$	0.09	$C_{27}H_{30}O_{16}$	301.03412	Rutin	Flavonoids	✓	✓	✓	✓	12
29	21.541	$[M - H]^-$	-0.07	$C_{21}H_{20}O_{10}$	311.05615, 341.06815	Vitexin	Flavonoids	✓	✓	✓	✓	12
30	22.104	$[M - H]^-$	-0.09	$C_{21}H_{20}O_{11}$	107.01337	Kaempferol-7-O-glucoside	Flavonoids	✓	✓	✓	✓	25
31	22.963	$[M - H]^-$	0.49	$C_{27}H_{30}O_{15}$	285.04010, 151.00293	Kaempferol-3-O-rutinoside	Flavonoids	✓	✓	✓	✓	21
32	23.618	$[M - H]^-$	-0.05	$C_{21}H_{20}O_{11}$	447.09378, 285.03928, 284.03265, 256.03519, 255.02997, 227.03502	Astragalgin	Flavonoids	✓	✓	✓	✓	26
33	23.975	$[M + H]^+$	0.3	$C_{16}H_{12}O_7$	153.01845	Isorhamnetin	Flavonoids	✓	✓	✓	✓	27





Table 1 (Contd.)

No	RT [min]	Reference ion $m/z$	Diff. (ppm)	Formula	Fragmentations ( $m/z$ )	Identification	Classification	Flower	Leaf	Stem	Root	References
34	24.036	$[M-H]^{-1}$	-0.18	$C_{21}H_{20}O_{10}$	151.00351, 239.03543, 240.04247, 268.03769, 432.10159	Apigenin-7-O- $\beta$ -D-glucoside	Flavonoids	✓	✓	✓	✓	6
35	24.699	$[M-H]^{-1}$	-2.32	$C_7H_6O_4$	109.02934, 81.03450	3,4-Dihydroxybenzoic acid	Organic acids	✓	✓	✓	✓	13
36	25.73	$[M-H]^{-1}$	-0.47	$C_9H_{10}O_5$	121.02936	Syringic acid	Organic acids	✓	✓	✓	✓	12
37	26.892	$[M-H]^{-1}$	-0.19	$C_{15}H_{20}O_4$	219.13885, 151.07648	( $\pm$ )-Abscisic acid	Sesquiterpenoids	✓	✓	✓	✓	28
38	28.722	$[M-H]^{-1}$	-0.17	$C_{15}H_{10}O_7$	273.04022, 178.99855, 151.00357	Quercetin	Flavonoids	✓	✓	✓	✓	12
39	28.741	$[M-H]^{-1}$	-0.06	$C_{15}H_{10}O_6$	241.05049, 217.05031, 199.03995	Luteolin	Flavonoids	✓	✓	✓	✓	12
40	30.967	$[M-H]^{-1}$	-0.14	$C_{15}H_{12}O_5$	271.06113, 177.01920, 151.00354, 119.05003, 107.01367, 93.03434	Naringenin	Flavonoids	✓	✓	✓	✓	29
41	31.834	$[M-H]^{-1}$	-0.08	$C_{15}H_{10}O_5$	225.05576, 201.05618, 181.06602, 151.00360, 117.03442	Apigenin	Flavonoids	✓	✓	✓	✓	18
42	32.07	$[M-H]^{-1}$	0.06	$C_{15}H_{10}O_6$	257.0444	Kaempferol	Flavonoids	✓	✓	✓	✓	19
43	33.683	$[M+H]^{+1}$	0.23	$C_{18}H_{30}O_2$	67.05460, 81.07015, 95.08580, 279.23236, 109.10146	$\alpha$ -Linolenic acid	Organic acids	✓	✓	✓	✓	30
44	39.864	$[M+H]^{+1}$	0.14	$C_{16}H_{14}O_5$	163.03407, 147.04419	Sakuranetin	Flavonoids	✓	✓	✓	✓	31
45	42.102	$[M+H]^{+1}$	-0.18	$C_{17}H_{21}NO_3$	161.05951, 135.04419	Piperanine	Alkaloids	✓	✓	✓	✓	32
46	43.114	$[M+H]^{+1}$	-0.48	$C_{17}H_{19}NO_3$	201.05476, 171.04424, 135.04417, 115.05435	Piperine	Alkaloids	✓	✓	✓	✓	32
47	65.446	$[M+H]^{+1}$	0.5	$C_{18}H_{30}O_2$	261.22183, 209.05350, 173.13307, 137.13290, 123.1170, 109.10140, 95.08575, 81.07012, 67.05458	$\alpha$ -Eleostearic acid	Organic acids	✓	✓	✓	✓	31
48	67.219	$[M+H]^{+1}$	0.11	$C_{30}H_{48}O_3$	95.08572, 107.08567	Oleanolic acid	Triterpenoids	✓	✓	✓	✓	33
49	67.271	$[M+H]^{+1}$	-0.07	$C_{30}H_{48}O_3$	411.36234, 393.35101	Ursolic acid	Triterpenoids	✓	✓	✓	✓	34
50	85.537	$[M-H]^{-1}$	0.09	$C_{30}H_{48}O_2$	439.35828, 421.34726	Roburic acid	Triterpenoids	✓	✓	✓	✓	35

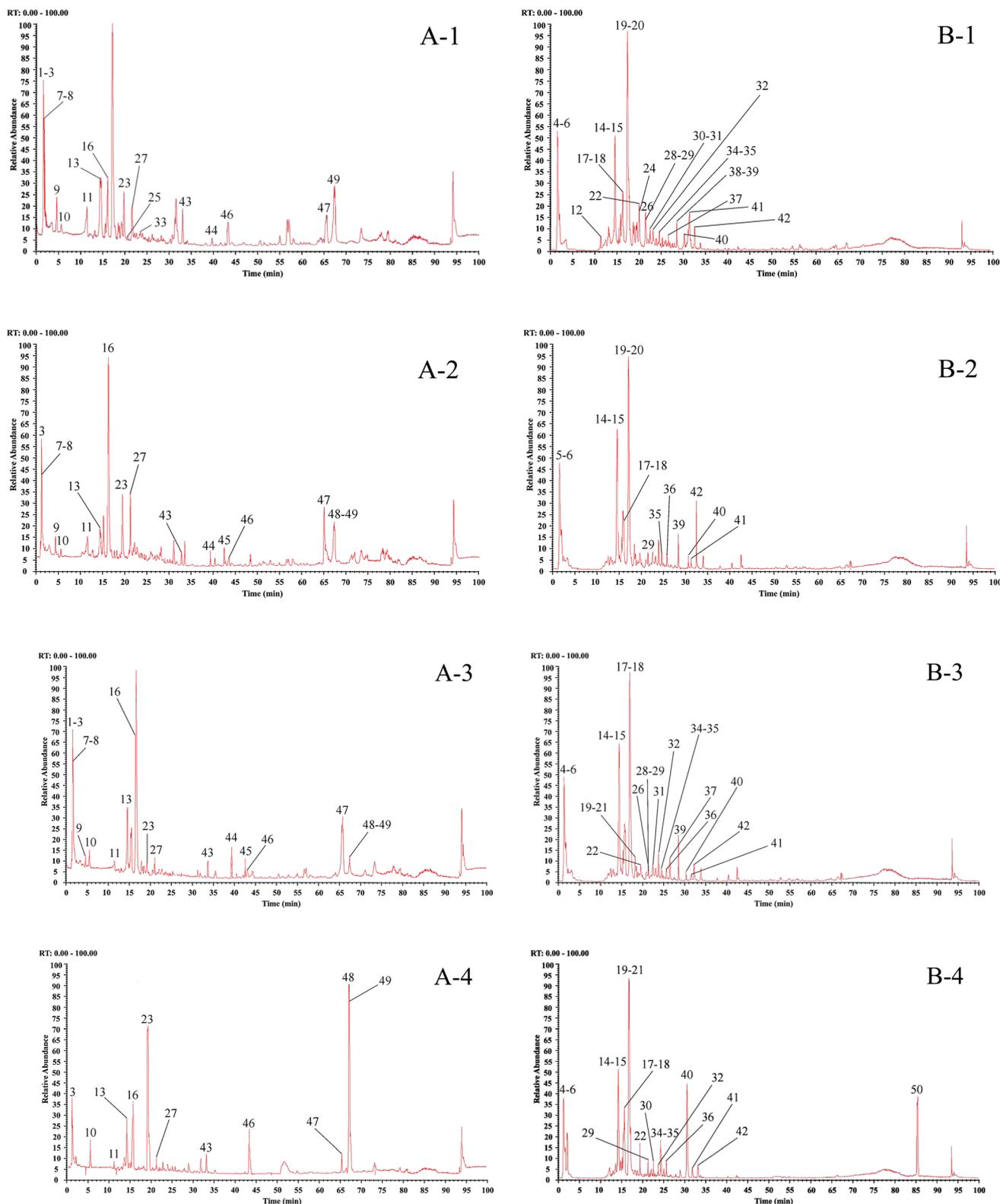


Fig. 2 The total ion current (TIC) chromatograms of various parts (1: flower; 2: leaf; 3: stem; 4: root) of *Gentiana macrophylla* Pall. in positive (A) and negative (B) modes.

$\text{CH}_5\text{N}_3^+$ . The cleavage pathway of this compound was consistent with literature reports,<sup>5</sup> and compound 1 was identified as DL-arginine (additional file 5: Fig. S5†).

**3.1.1.5 Identification of alkaloids.** These compounds are easy to cleave between carbon-carbon single bond and carbon-carbon double bond, easy to lose piperidine, and then continue

to lose one molecule of CO. Taking peak 46 as an example, the molecular ion peak of  $m/z$  286.14363  $[M + H]^+$  was detected in the positive ion mode, and  $C_5H_{11}N$  was lost to form a fragment ion of  $m/z$  201.05476, and CO was lost to form an ion fragment of  $m/z$  171.04424, and ion fragments of  $m/z$  135.04417  $[C_8H_6O_2 + H]^+$ . Compound 46 was confirmed to be piperine based on the molecular formula, cleavage rule, fragment information and comparison results of compounds in the literature (additional file 6: Fig. S6†).<sup>39</sup>

**3.1.2 A comparative chemical composition of various parts of *G. macrophylla*.** Among the detected compounds, the flower, leaf, stem, and root of *G. macrophylla* contained 45, 32, 42, and 32 compounds, respectively. The distribution of chemical composition in various parts of *G. macrophylla* was analyzed using venny 2.1.0 (Fig. 3). As Fig. 3 shows, the common components of the four parts were amino acids (compounds 3, 10, 11, and 13), organic acids (compounds 15, 17, 18, 35, 43, and 47), iridoids (compounds 14, 16, 19, 20), triterpenoids (compound 49), flavonoids (compounds 23, 37, 29, 40, 41, and 42), and alkaloids (compound 46), respectively. Common compounds of flowers, stems, and leaves were amino acids (compounds 7, 8, and 9), organic acids (compound 5), and flavonoids (compounds 39 and 40). The common compounds of stem and leaf roots were organic acids (compound 36) and triterpenoids (compound 48). The common compounds of flowers, leaves, and roots were organic acids (compound 4 and 22) and flavonoids (compounds 32 and 34). The common compounds of flowers and stems were amino acids (compounds 1 and 2), organic acids (compound 26), sesquiterpenoids (compound 37), and flavonoids (compounds 28 and 31). Compounds contained in stems and leaves was alkaloids (compound 45). The common compound to flowers and roots

were flavonoids (compound 30). Only in the flower, there were amino acids (compound 24), organic acids (compound 12), and flavonoids (compounds 25, 33, and 38). However, the compounds distributed only in the root had a flavonoids (compound 21) and a triterpenoids (compound 50).

The resulting UPLC-Q-orbitrap-MS data were uploaded into SIMCA 14.1 software and VIP values were screened using the area of the characteristic peak as a variable. The results show that the compounds 14 (loganin acid), 16 (swertiamarin), 20 (sweroside), and 29 (vitexin) became key markers due to their high VIP scores. In addition, moreover, according to UPLC-Q-orbitrap-MS data, the peak area values of 19 (gentiopicroside) and 23 (isoorientin) were high and the Fig. 3 also showed that these two components were the key components common to all four fractions, and 50 (roburic acid) was found exclusively in the root as a triterpenoid component. Therefore, we will pay attention to these seven compounds (Fig. 4) in the next characterization chromatography experiments.

### 3.2 A comparative the characteristic chromatogram of various parts of *G. macrophylla* by HPLC

A diagram of the solution of the mixed standards determined according to the chromatographic conditions was shown in Fig. 5. Ten batches of flowers (S1–S10), ten batches of leaves (S11–S20), ten batches of stems (S21–S30), and ten batches of roots (S31–S40) of *G. macrophylla* were injected. S1 was selected as the reference map, and the time window width was set as 0.2 min. Multi-point correction and Mark peak matching were performed on chromatographic peaks, and ten batches of characteristic map matching maps of flowers, leaves, stems, and roots of *G. macrophylla* were generated, as well as control fingerprint maps (Fig. 6). Gentiopicroside (Pick 7) was selected

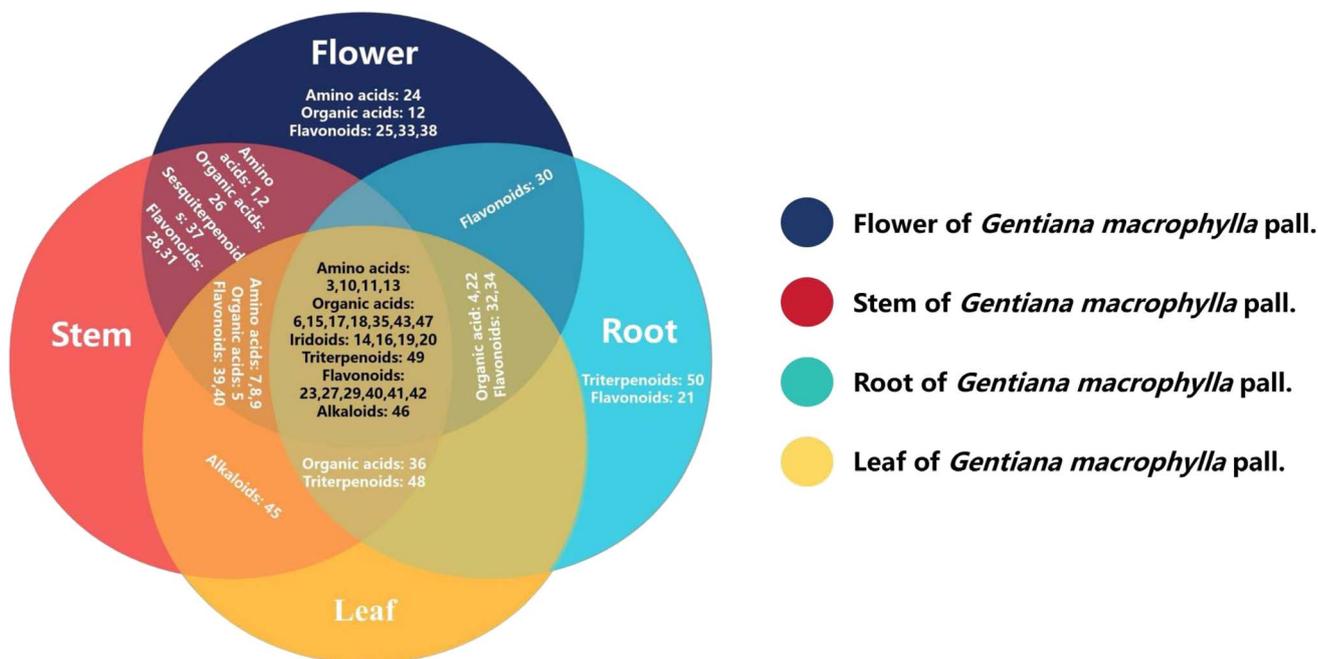


Fig. 3 The analysis of the chemical composition distribution of UPLC-Q-Exactive MS data.



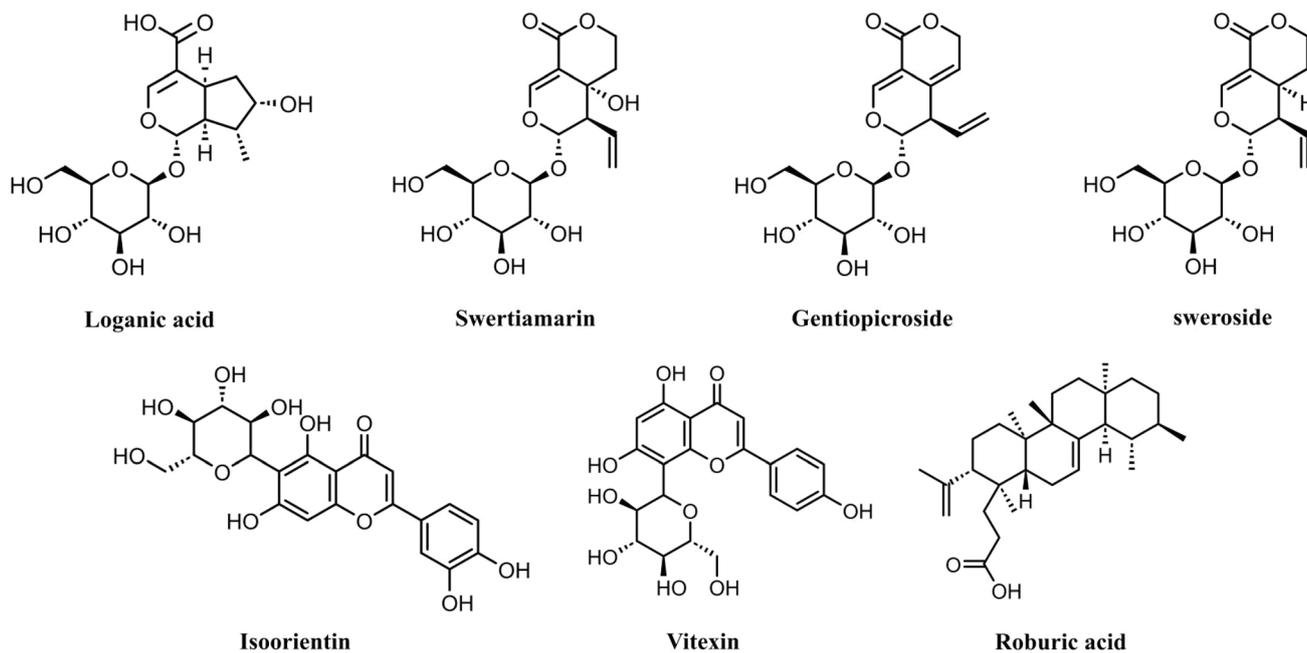


Fig. 4 Chemical structure diagram of seven characteristic components.

as the reference peak because of its good separation and intermediate retention time in the characteristic chromatogram of various parts of *G. macrophylla*. The similarity of the S1–S40 characteristic chromatogram and the control chromatogram were all greater than 0.9 (additional file 7: Table S1†). This indicated that the chemical composition of each batch of samples was stable. From the characteristic chromatogram, it can be seen that there were 11 characteristic peaks in various parts of *G. macrophylla*. In order to better observe the differences between groups and reduce the error of the results, PCA analysis (Fig. 7A) and supervised mode PLS-DA identification analysis (Fig. 7B) were performed with the peak areas of 11 characteristic peaks in the 40 batches of characteristic chromatogram as variables. The components with large contributions to the differences between groups were selected to classify the 40 batches of *G. macrophylla*. The PLS-DA models  $R^2X$ ,  $R^2Y$ , and  $Q^2$  were all  $>0.5$  (Fig. 7C), indicating that the model had

good stability and predictive ability. VIP value is the key indicator for screening quality difference markers, which reflects the contribution of common peaks to the difference between groups. When the threshold of VIP is set to be greater than 1, four quality difference markers are screened, including common peaks 8, 6, 7, and 5, as shown in Fig. 7D. VIP values were 1.45037, 1.08101, 1.02542, and 1.00016, respectively. Additionally, the retention times of the 11 characteristic peaks of the characterisation chromatogram and the mixed standards were compared, and four characteristic peaks were identified, including loganic acid, swertiamarin, gentiopicroside, and sweroside. From these two aspects, it can be found that these components play a significant role in the quality differences among batches of samples from various parts.

Combining the experimental data determined by the UPLC-Q-orbitrap MS method with the characteristic chromatogram, we can know that loganic acid, swertiamarin, gentiopicroside

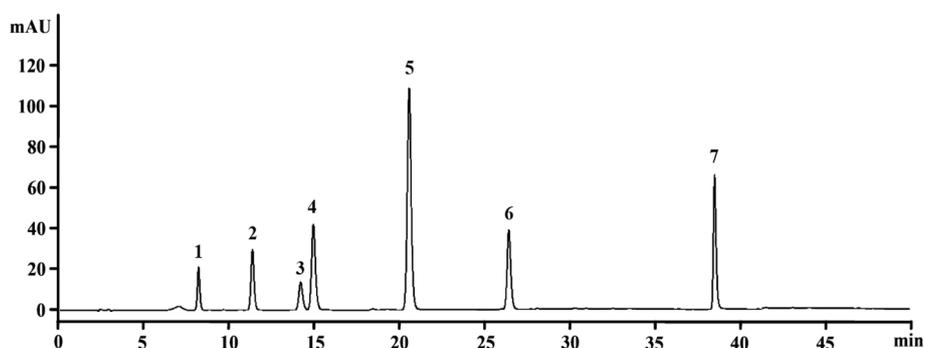


Fig. 5 The mixed standards (1: Loganin acid; 2: Swertiamarin; 3: Gentiopicroside; 4: Sweroside; 5: Isoorientin; 6: Vitexin; 7: Apigenin) of *G. macrophylla*.

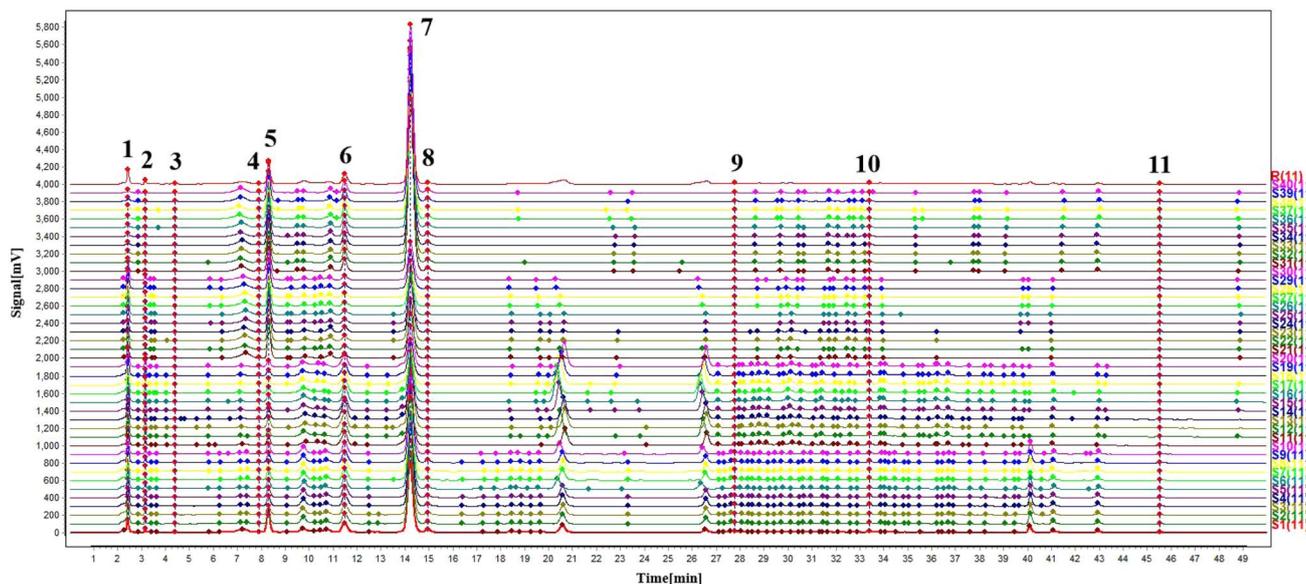


Fig. 6 The characteristic chromatogram (5: loganic acid; 6: swertiamarin; 7: gentiopicroside; 8: sweroside) of various parts (S1–S10: flower; S11–S20: root; S21–S30: stem; S31–S40: leaf; R: control) of *G. macrophylla*.

and sweroside are the components common to the four parts, isoorientin and vitexin are present in the flowers, stems, and leaves, while roburic acid is only present in the roots. For *G.*

*macrophylla*, the components of iridoids and triterpenoids represented by loganic acid, swertiamarin, gentiopicroside, sweroside and roburic acid have become the main quality

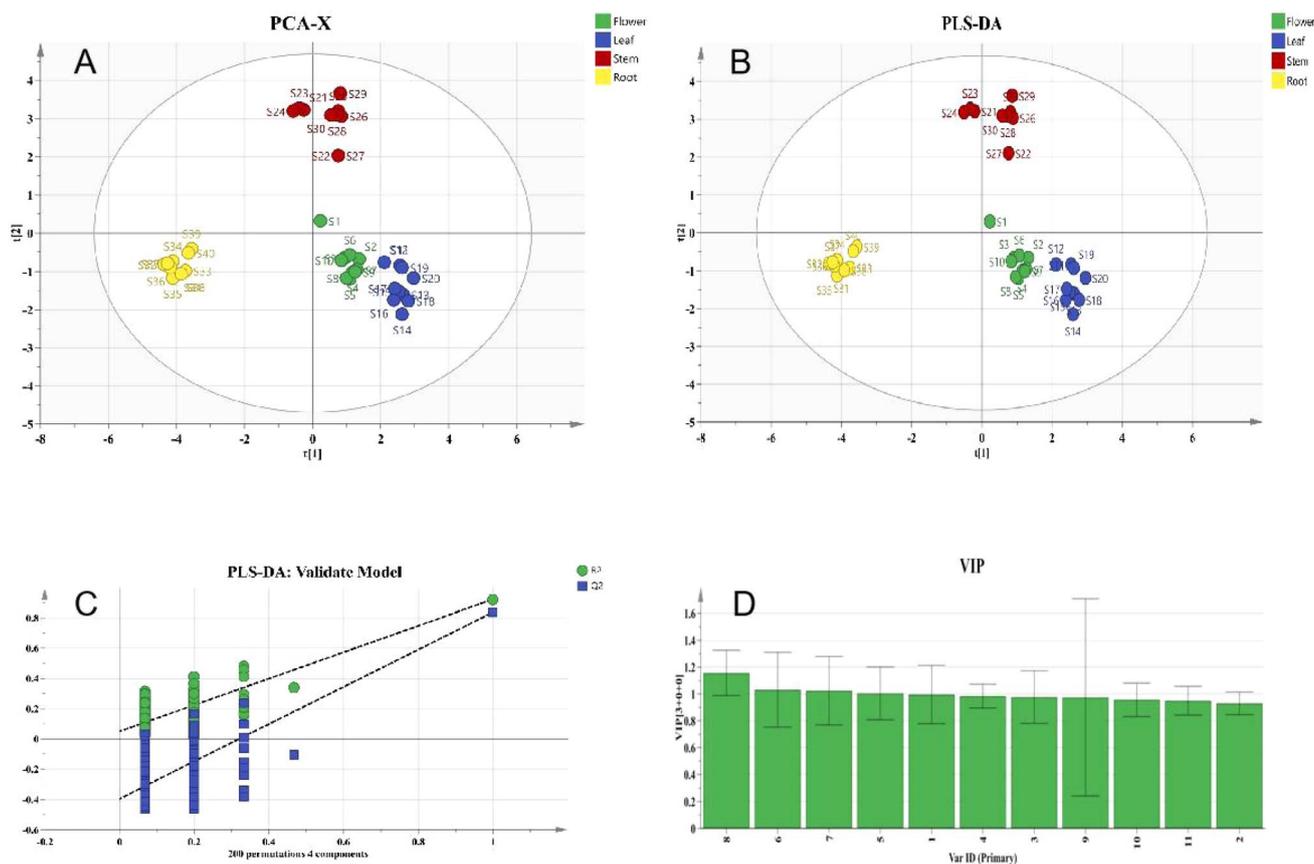


Fig. 7 PCA (A), PLS-DA (B), PLS-DA permutation test (C), VIP (D) diagram of 40 batches of *G. macrophylla*.



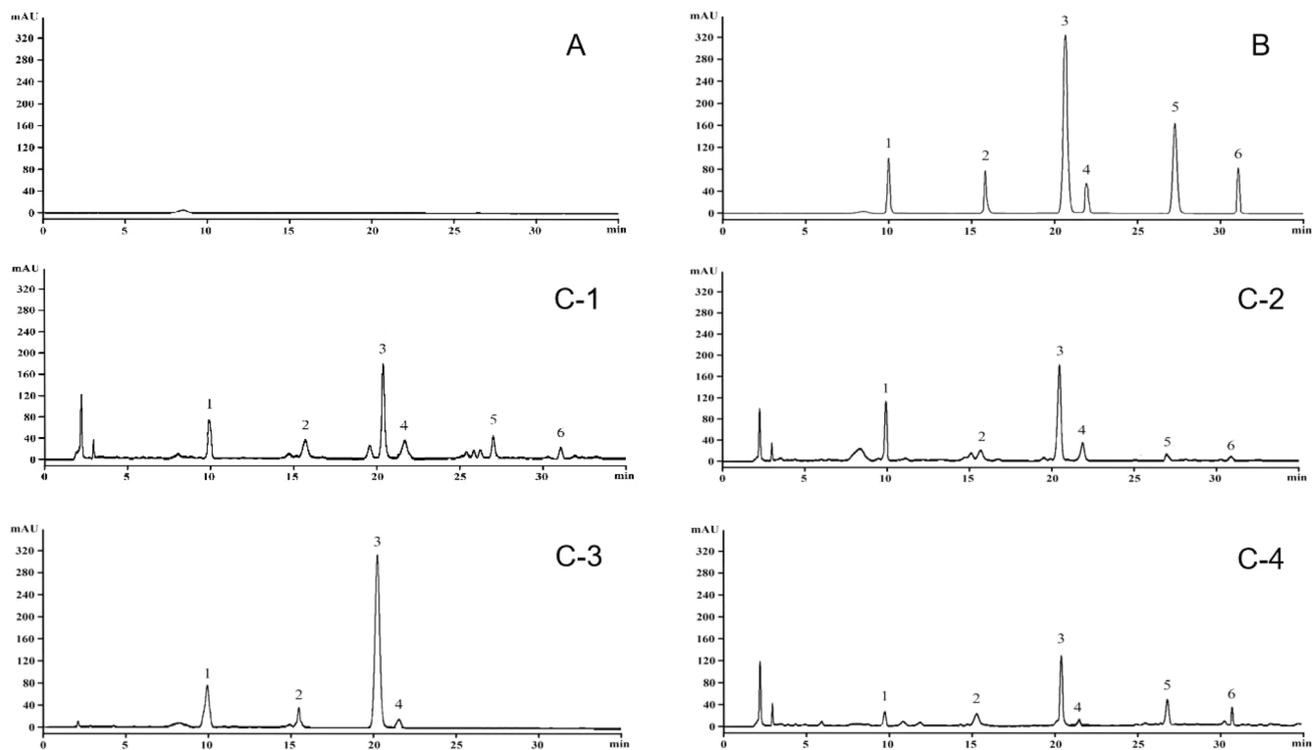


Fig. 8 The HPLC chromatogram (A: blank control; B: mixed standards; C: sample) of iridoids and flavonoids (1. Loganic acid; 2. Swertiamarin; 3. Gentiopicroside; 4. Sweroside; 5. Isoorientin; 6. Vitexin) from various parts (1. Flower; 2. Stem; 3. Root; 4. Leaf) of *G. macrophylla*.

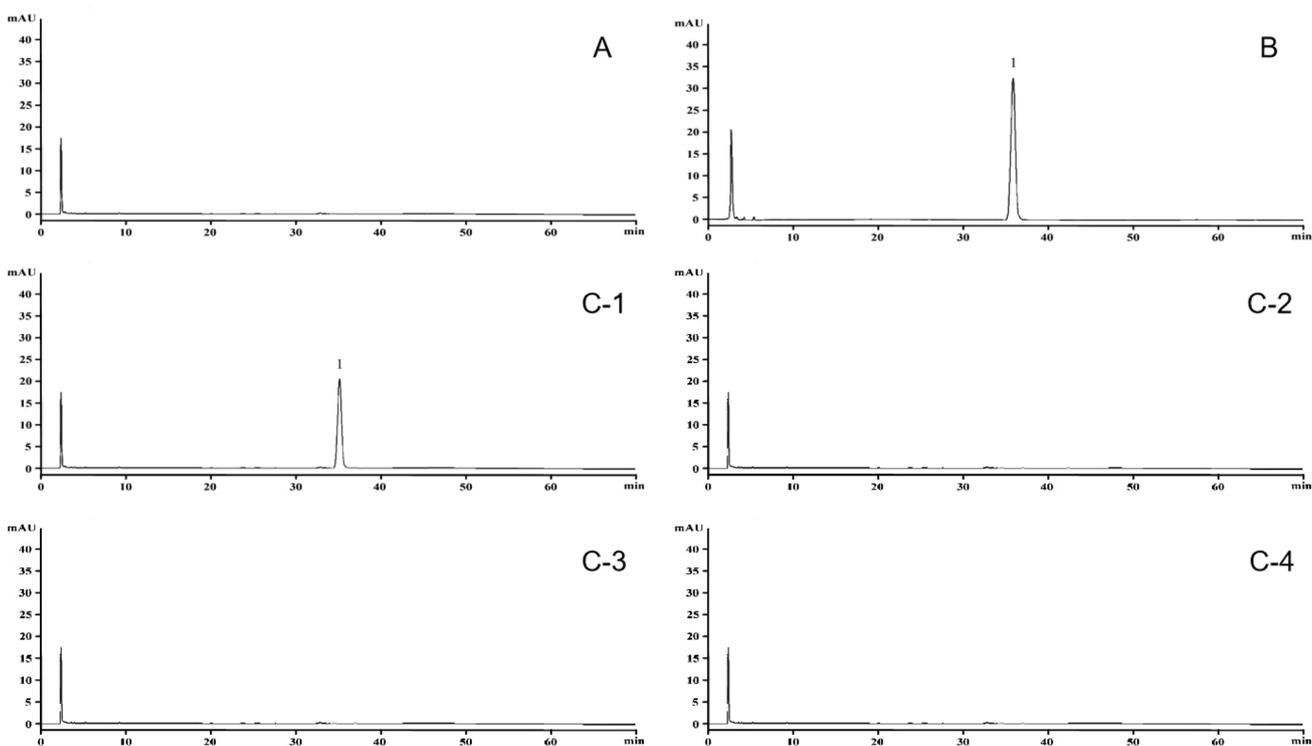


Fig. 9 The HPLC chromatogram (A: blank control; B: mixed standards; C: sample) of triterpenoids (1. Roburic acid) from various parts (1. Stem; 2. Flower; 3. Root; 4. Leaf) of *G. macrophylla*.



Table 2 The calibration curves of the HPLC analysts

Analytes	Linear equation	<i>r</i>	Range ( $\mu\text{g mL}^{-1}$ )	LOD ( $\mu\text{g mL}^{-1}$ )	LOQ ( $\mu\text{g mL}^{-1}$ )
Loganic acid	$y = 11.936x - 41.367$	0.9998	11.6–184.9	3.27	11.47
Swertiamarin	$y = 15.509x - 44.846$	0.9995	9.8–156.6	2.56	9.77
Gentiopicroside	$y = 11.160x - 233.24$	0.9995	77.6–1242.0	16.03	61.16
Sweroside	$y = 17.839x - 19.883$	0.9996	3.4–54.2	1.24	2.72
Isoorientin	$y = 30.318x - 93.675$	0.9995	9.6–153.6	8.09	9.51
Vitexin	$y = 14.610x + 3.0167$	0.9998	7.0–111.5	3.09	6.79
Roburic acid	$y = 7912.3x - 2.7375$	0.9997	22.3–356.6	1.71	5.70

control components of its roots in the pharmacopoeia of the People's Republic of China. And these components are the characteristic components shared by the same genus of plants in Gentianaceae, and most of the research reports. The root is

abundant in iridoids and triterpenoids, components that align closely with its traditional effects, including dispelling wind and dehumidification and clearing heat of deficiency and dampness. For example, the swertiamarin could inhibit the

Table 3 The recovery of the HPLC analysts

Analytes	Sample (g)	Original (mg)	Spiked (mg)	Found (mg)	Recovery (%)	Average (%)	RSD (%)
Loganic acid	0.0102	0.11	0.18	0.32	112.03	109.46	1.61
	0.0104	0.11	0.18	0.32	113.84		
	0.0101	0.11	0.18	0.31	110.44		
	0.0101	0.11	0.18	0.31	105.87		
	0.0104	0.11	0.18	0.31	106.81		
	0.0103	0.11	0.18	0.31	107.77		
Swertiamarin	0.0102	0.09	0.10	0.22	123.52	115.83	1.64
	0.0104	0.09	0.10	0.21	114.75		
	0.0101	0.09	0.10	0.21	109.13		
	0.0101	0.09	0.10	0.22	121.42		
	0.0104	0.09	0.10	0.21	109.32		
	0.0103	0.09	0.10	0.22	116.85		
Gentiopicroside	0.0102	1.41	1.40	3.11	121.36	116.74	1.17
	0.0104	1.44	1.40	3.00	111.90		
	0.0101	1.40	1.40	2.98	113.41		
	0.0101	1.40	1.40	3.09	121.22		
	0.0104	1.44	1.40	3.06	115.99		
	0.0103	1.42	1.40	3.05	116.58		
Sweroside	0.0102	0.06	0.08	0.15	109.80	108.76	1.80
	0.0104	0.06	0.08	0.15	110.26		
	0.0101	0.06	0.08	0.15	111.81		
	0.0101	0.06	0.08	0.14	107.61		
	0.0104	0.06	0.08	0.15	106.14		
	0.0103	0.06	0.08	0.15	106.92		
Isoorientin	0.0102	0.08	0.09	0.19	119.09	117.79	1.23
	0.0104	0.09	0.09	0.19	119.14		
	0.0101	0.08	0.09	0.19	119.12		
	0.0101	0.08	0.09	0.18	115.73		
	0.0104	0.09	0.09	0.19	116.78		
	0.0103	0.08	0.09	0.19	116.85		
Vitexin	0.0102	0.07	0.09	0.18	110.36	108.41	1.49
	0.0104	0.08	0.09	0.17	103.62		
	0.0101	0.07	0.09	0.18	111.30		
	0.0101	0.07	0.09	0.18	109.38		
	0.0104	0.08	0.09	0.18	112.09		
	0.0103	0.08	0.09	0.17	103.74		
Roburic acid	0.0209	0.2884	0.2389	0.53	102.06	103.94	1.30
	0.0204	0.2815	0.2389	0.52	101.40		
	0.0207	0.2857	0.2389	0.54	107.02		
	0.0206	0.2843	0.2389	0.54	105.69		
	0.0201	0.2774	0.2389	0.53	104.14		
	0.0208	0.2870	0.2389	0.53	103.32		



expression of NF- $\kappa$ B P65, p-I $\kappa$ B $\alpha$ , p-JAK2 and p-STAT3 proteins at both the whole animal and cellular levels, confirming that swertiamarin may produce anti-inflammatory effects through the NF- $\kappa$ B/I $\kappa$ B and JAK2/STAT3 signalling pathways.<sup>40</sup> Roburic acid has been shown to regulate the nuclear factor kappa-B (NF- $\kappa$ B) and mitogen-activated protein kinase (MAPK) pathways, inhibit the secretion of pro-inflammatory factors nitric oxide and IL-6, thereby exerting anti-inflammatory effects.<sup>41</sup> It is worth noting that the significant enrichment of flavonoids in flowers, stems and leaves expands the development potential of non-traditional medicinal parts. Research has demonstrated that isoorientin efficiently scavenges DPPH free radicals and protects hepatocytes from H<sub>2</sub>O<sub>2</sub>-induced cellular dysfunction, mitigating oxidative stress-induced cellular damage.<sup>42,43</sup> Isoorientin pretreatment effectively inhibits the synthesis of IL-8 and MMP-1, suppressing inflammation and inhibiting the progression of inflammatory diseases.<sup>44</sup> The antioxidant and anti-inflammatory properties of flavonoids contained in flowers provide a modern interpretation of the traditional effects associated with the removal of “Xieriwusu” (“Xieriwusu” means rheumatism in Mongolian medicine).

### 3.3 A comparative main component content of various parts of *G. macrophylla* by HPLC

The results of UPLC-Q-orbitrap MS analysis and characteristic chromatogram analysis showed that seven components were differentially expressed, including loganic acid, swertiamarin, gentiopicroside, sweroside, isoorientin, vitexin, and roburic acid. The ultraviolet (UV) detection is at the end of the absorption because the conjugated double bond of roburic acid is so not much. If roburic acid is analyzed under the same chromatographic conditions as the other six components, its peak area will be markedly diminished, thereby potentially affecting the results. To accurately determine the aforementioned seven components, this experiment utilizes two distinct chromatographic conditions, which were validated through preliminary experiments.

Through HPLC analysis, from the results of the two specificity experiments (Fig. 8 and 9), it was known that there were chromatographic peaks with the same retention time on the

chromatogram of the mixed standard solution and the sample solution. The separation of each component was greater than 1.5, and there was no interference with the blank control solution. This indicates that the established HPLC method has good specificity. Linear regression was performed using the peak area of each component as the ordinate and the reference product concentration (mg mL<sup>-1</sup>) as the abscissa to obtain the regression equation and correlation coefficient ( $r$ ) to determine the linear range. The limit of detection (LOD) and limit of quantification (LOQ) were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively. The standard curve, linear range, LOD, and LOQ were shown in Table 2. There was a good linear relationship between the detected mass concentration and the peak area ( $r \geq 0.9995$ ). The precision and stability results showed that RSD values of the six compounds were all less than 2.0% (additional file 8: Table S2†), indicating that the method was sensitive, accurate, and reliable. By precisely adding standards in a 1 : 1 content ratio to previously examined samples of known content, the recovery was ascertained (Table 3). The table shows that the average recovery rates were 109.46%, 115.83%, 116.74%, 108.76%, 117.79%, 108.41% and 103.94%, respectively. The outcomes demonstrate that this approach has an excellent recovery rate. HPLC content determination data were analyzed in Table 4.

The data from the HPLC content measurements were analysed by the horizontal stack bar and error bar (Fig. 10), and the results were compared. The comparison revealed that, the content of iridoids such as loganic acid, swertiamarin, gentianoside, and sweroside was highest in the roots, where they were more than twice as abundant as in the flowers, stems, and leaves. In addition, roburic acid, which was exclusively found in the roots, belongs to the triterpenoids. While the flavonoid components are more highly distributed in the flowers, stems and leaves. This further confirms that the iridoids represented by gentianoside and the triterpenoids represented by roburic acid can be used as quality markers for *G. macrophylla* roots, while the flavonoids represented by isoorientin and vitexin can be used as quality markers for its flowers, stems and leaves. In another way, through this experiment we can learn that all four parts of the *G. macrophylla* flower have medicinal value and all will be able to be used as medicinal parts.

Table 4 Comparison of the contents determined by calibration curve<sup>a</sup>

Analytes	Average powder content (mg g <sup>-1</sup> )				Average extract content (mg g <sup>-1</sup> )			
	Flower	Leaf	Stem	Root	Flower	Leaf	Stem	Root
Loganic acid	2.21	1.06	8.72	13.94	10.91	6.84	31.42	32.55
Swertiamarin	2.47	1.60	1.21	6.20	9.03	8.28	5.62	13.50
Gentiopicroside	38.35	18.07	17.87	128.10	138.24	78.04	76.70	298.48
Sweroside	1.81	0.13	0.91	2.05	6.04	1.00	4.09	4.44
Isoorientin	1.80	3.82	0.26	—	8.19	17.88	2.30	—
Vitexin	2.02	4.69	0.39	—	7.35	32.81	1.39	—
Roburic acid	—	—	—	3.60	—	—	—	9.10

<sup>a</sup> For the content determination experiment, three batches of four various parts were determined, the peak areas were recorded in turn, the content was calculated, and the final results were averaged.



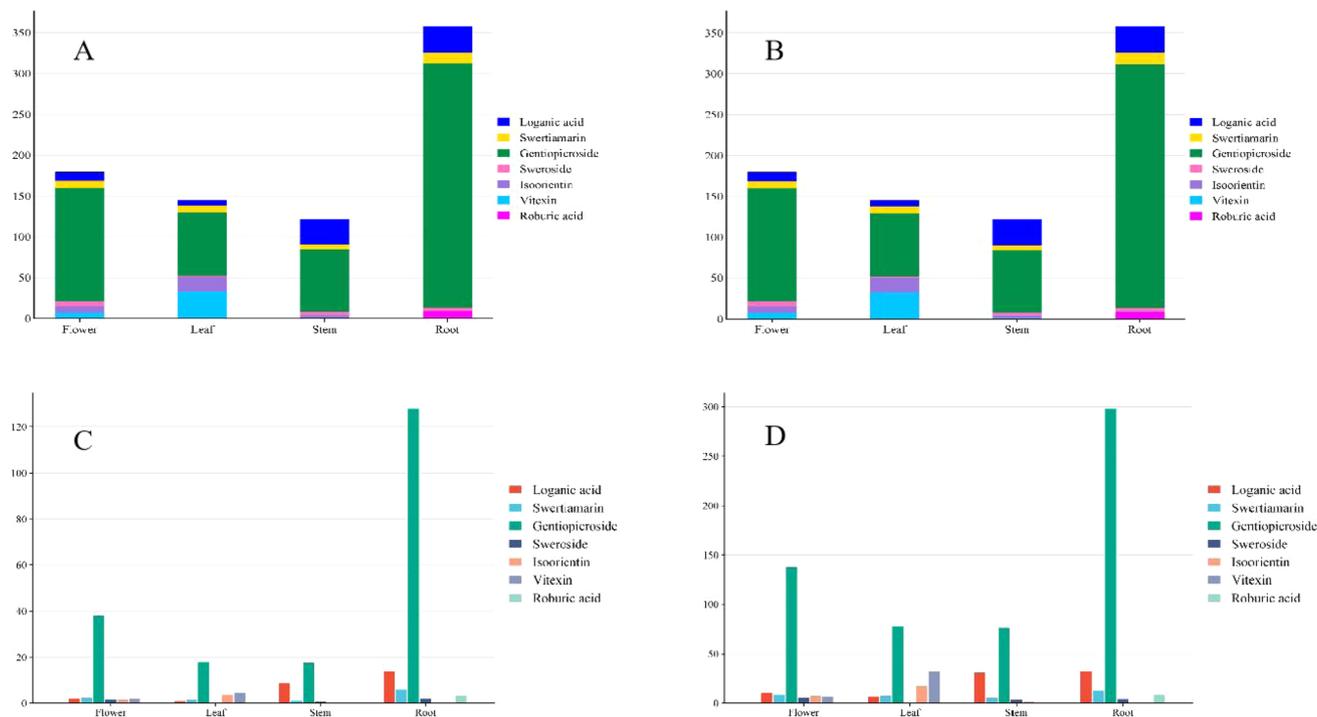


Fig. 10 Horizontal stack bar of the contents of seven components in powder (A) and extract (B) *G. macrophylla*, error bar of comparison of the contents of seven components in powder (C) and extract (D) among the *G. macrophylla* of the various parts.

## 4. Conclusion

In this paper, we investigated the chemical constituents of four parts of *G. macrophylla* by UPLC-Q-orbitrap MS analysis combined with comparative characteristic chromatogram. On this basis, we then determined the content of its characteristic components. We found that the roots of *G. macrophylla* are rich in iridoids represented by gentianoside, and triterpenoids represented by roburic acid, while flavonoids represented by isoorientin and vitexin are mainly found in the flowers, stems, and leaves of *G. macrophylla*, and their contents are relatively high. From the modern pharmacological effects of these chemical components, the traditional efficacy of *G. macrophylla*, and the results of our study, we concluded that *G. macrophylla*, as a medicinal plant, has roots, flowers, leaves, and stems that can be parts with medicinal value, and that iridoids represented by gentianoside and triterpenoids represented by roburic acid can be used as quality markers for the roots of *G. macrophylla*, and the flavonoids represented by isoorientin and vitexin can be the quality markers of its flowers, stems and leaves. In summary, this study not only lays a scientific foundation for the quality control of this herb, but also provides a favorable basis for expanding the development and utilization of its non-traditional medicinal parts.

## Data availability

Data will be made available on request.

## Author contributions

Qier Mu: writing-original draft, methodology, investigation, data curation, conceptualization. Yanqiu Bai: investigation, data curation. Junni Qi: formal analysis, data curation. Chula Sa: writing-review & editing, supervision, data curation, conceptualization.

## Conflicts of interest

The Authors declare no conflict of interest.

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