Research article

Comparative study on chemical constituents of different medicinal parts of *Lonicera japonica* Thunb. Based on LC-MS combined with multivariate statistical analysis

Xinrui Zhang [a], Xiao Yu [b], Xiaomei Sun [a], Xianbo Meng [c], Jian Fan [b], Fang Zhang [a,*], Yongqing Zhang [a,**]

[a] *College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, 250355, China*
[b] *Shandong Medicine Technician College, Taian, 271016, China*
[c] *Zibo Institute for Food and Drug Control, Zibo, 255035, China*

ABSTRACT

Lonicerae japonicae flos (LJF), Lonicerae japonicae caulis (LJC), Lonicerae folium (LF) and Lonicerae fructus (LFR) are derived from *Lonicera japonica* Thunb., which are formed due to different medicinal parts. The efficacy of the 4 medicinal materials has similarities and differences. However, little attention has been paid to illustrate the differences in efficacy from the perspective of phytochemistry. In this study, ultra-high performance liquid chromatography coupled with hybrid quadrupole-orbitrap mass spectrometry (UPLC-Q-Exactive-Orbitrap-MS) was used to qualitatively analyze the ingredients in 4 herbs. A total of 86 compounds were plausibly or unambiguously identified, there were 54 common components among the 4 medicinal materials, and each kind of medicinal materials had its own unique components. On the basis of qualitative analysis, ultra-performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (UPLC-QQQ-MS/MS) was used to quantitatively analyze 31 components contained in 4 medicinal materials, and principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA) and other multivariate statistical analysis were furtherly performed for comparing the component contents. The results showed that the samples from the same parts were clustered into one group, and the samples from different medicinal parts were significantly different. The analysis of variable importance projection (VIP) value of the OPLS-DA model showed that 10 components including chlorogenic acid, secologanic acid, isochlorogenic acid A, loganin, lonicerin, loganic acid, secoxyloganin, sweroside, luteolin and rhoifolin were the main difference components among the 4 medicinal materials. The study not only lays a solid foundation for the intrinsic quality control of 4 medicinal materials and the study of different effects of the 4 medicinal materials at the phytochemical level, but also provides a basis for more rational utilization of various parts of *L. japonica* and expansion of medicinal resources.

* Corresponding author.
** Corresponding author.
E-mail addresses: zfang_819@163.com (F. Zhang), zyq622003@126.com (Y. Zhang).

1. Introduction

It is a common phenomenon that different herbal materials are formed owing to being derived from different parts of the same medicinal plant [1]. Lonicerae japonicae flos (LJF), Lonicerae japonicae caulis (LJC), Lonicerae folium (LF) and Lonicerae fructus (LFR) are representative ones, which were derived from *Lonicera japonica* Thunb [2–5]. LJF is originated from the dried buds and flowers, LJC is derived from the dried stems, LF is derived from the dried leaves and LFR is originated from the dried mature fruits. The four herbal materials all possess the effect of heat-clearing and detoxifying, but each has its own advantages, of which LJF is good at dispersing wind heat, and can be used for the treatment of wind-heat cold, throat impediment, erysipelas and so on; LJC is good at dredging wind and clearing collaterals, which can be used for the treatment of wind-damp-heat arthralgia, joint swelling and heat pain; LF mainly treat seasonal febrile disease fever, heat toxin blood dysentery, infective hepatitis, carbuncle and swelling toxin; LFR has the effect of detoxifying and stopping dysentery, and is used for the treatment of heat sore swelling toxin and dysentery. But until now, the reason for the difference in efficacy of 4 herbal materials has not yet been revealed.

Chemical components are the material basis of the pharmacodynamics of Traditional Chinese medicines [6]. Many studies have shown that the differences in composition or content of chemical components is the main reason for the difference in efficacy of different herbal materials formed due to different medical parts of the same medical plant [7,8]. In the past decades, a large number of literature about *L. japonica* have been reported, which focused on identifying chemical compounds, quality control, and so on [9–12]. More than 200 compounds were isolated and identified from *L. japonica*, such as phenolic acids, flavonoids, triterpenoid saponins and iridoids. Several reports concentrated predominantly upon quantitative or qualitative comparison between two or among three medical parts [1,13–15], but no research focusing on a comprehensive comparison of chemical components in 4 medical parts of *L. japonica* was reported.

LJF and LJC are listed in calendar edition of Chinese Pharmacopoeia (ChP) with the name "jin-yin-hua" and "ren-dong-teng", but LF and LFR have not received enough attention. As a by-product of LJF pruning process, the yield of the leaves is about 10 times that of the flower [16]. Modern pharmacological studies have demonstrated that LF have antibacterial [17], antiviral [18], anti-inflammatory [19], antioxidant [20], and hepatoprotective [21]. LFR has the effects of antibacterial [22] and antioxidant [23].

In contrast with LJF and LJC, the research on LF and LFR is relatively lagging behind, in particular, the research on the chemical components only focused on one or several bioactive compounds [17,22], which was one-sided. Therefore, for accelerating the development and utilization of LF and LFR, comprehensive analysis on chemical components should be conducted.

In this study, a novel UPLC-Q-Exactive-Orbitrap-MS analysis method was established for comprehensively identifying and comparing the chemical compositions in four medical parts. And then, a sensitive and practical UPLC-QQQ-MS/MS method was developed for simultaneously determining the contents of 31 components, including 9 phenolic acids, 14 flavonoids and 8 iridoid glycosides (Fig. A.1) in 33 batches of LJF, LJC, LF and LFR. In addition, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed to distinguish the samples and reveal the differential compositions among 4 medical parts according to the amount of ingredient being tested. This study presented the first report on qualitative analysis and quantitative comparison of chemical components contained in LJF, LJC, LF and LFR and found differential chemical compositions in 4 medical parts, which would not only lay a foundation for revealing the formation mechanism of the differences in the efficacy of 4 herbal medicines from the perspective of chemical composition, but also provide a basis for more rational utilization of various parts of *L. japonica* and expansion of medicinal resources.

2. Materials and methods

2.1. Plant materia

The *L. japonica* planted in the Medicinal Plants of Shandong University of Traditional Chinese Medicine were labeled. In May, during the first flowering period, the flower buds were collected as LJF (S1–S9), in September, the annual branches (color is brown) and the mature leaves (dark green in color and more than 4 cm in length) were collected separately and used as LJC (S10–S18) and LF (S19–S27) respectively, and in December, the mature fruits of *L. japonica* (black in color) were collected as LFR (S28–S33), Fig. A.2. All the samples were authenticated by Prof. Fang Zhang of the Shandong University of Traditional Chinese Medicine. The voucher specimens were deposited at the Traditional Chinese Medicine Identification Laboratory of College of Pharmacy, Shandong University of Traditional Chinese Medicine.

2.2. Chemicals and reagents

The chemical standards of eriodictyol-7-O-glucoside (1), hyperoside (2), rutin (3), isoquercitrin (4), luteoloside (5), lonicerin (6), kaempferol-3-O-rutinoside (7), astragalin (8), narcissin (9), apigenin-7-O-glucoside (10), rhoifolin (11), luteolin (12), apigenin (13), diosmetin (14), protocatechuic acid (15), neochlorogenic acid (16), chlorogenic acid (17), cryptochlorogenic acid (18), isochlorogenic acid B (20), isochlorogenic acid A (22), isochlorogenic acid C (23), loganic acid (24), morroniside (25), secologanic acid (26), sweroside (27), loganin (28), and secoxyloganin (29) were purchased from Chengdu Push Biotechnology Co. Ltd. (Sichuan, China). Caffeic acid (19) and caffeic acid methyl ester (21) were purchased from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China). (E)-Aldosecologanin (30) and (Z)-Aldosecologanin (31) were purchased from Chem Faces Biochemical Co. Ltd. (Wuhan, China). The purities of all standards components were not less than 98 %, tested by HPLC analysis.

Formic acid of LC-MS grade was purchased from Thermo Scientific (Thermo Scientific, MA, USA), LC-MS grade methanol and

acetonitrile were supplied from Honeywell International Inc. (Muskegon, MI, USA). The deionized water was prepared by a Milli-Q water purification system (Billerica, MA, USA).

2.3. Sample solutions preparation

The sample solutions for qualitative analysis were prepared according to the references reported [24,25]. The samples powders (0.5 g) of LJF, LJC, LF and LFR, were accurately weighed and extracted by ultrasonication (200 W, 40 Hz) with 25 mL of 50 % methanol for 30 min. After being cooled to room temperature, the mixtures were weighed again, and the weights lost were replenished with 50 % methanol. And then, the samples were centrifuged at 12000 r/min for 10 min and supernatants were filtered through a 0.22 μm membrane prior to UPLC-Q-Exactive-Orbitrap-MS.

33 batches of samples including LJF, LJC, LF and LFR, were crushed into powder and screened through a 60-mesh sieve. The sample powder (0.25 g) was accurately weighed and then ultrasonically extracted with 20 mL of 75 % methanol for 60 min. After cooling to room temperature, the same solvent was added to compensate for the weight lost during extraction. Then the extract was centrifuged at 12,000 r/min for 10 min. Afterwards, the supernatant was filtered through a 0.22 μm membrane before UPLC-QQQ-MS/MS analysis.

2.4. Preparation of standard solutions

Each reference compound was accurately weighted and completely dissolved in suitable solvent to produce their respective stock solutions, and the concentration of 31 reference compounds were as follows: (1) 1.005, (2) 1.090, (3) 1.040, (4) 1.035, (5) 1.145, (6) 1.090, (7) 1.110, (8) 1.020, (9) 1.040, (10) 1.005, (11) 0.935, (12) 1.105, (13) 0.980, (14) 1.005, (15) 1.025, (16) 0.990, (17) 2.080, (18) 1.850, (19) 1.210, (20) 1.700, (21) 1.625, (22) 2.140, (23) 1.135, (24) 1.160, (25) 1.495, (26) 1.480, (27) 1.185, (28) 1.740, (29) 1.690, (30) 1.000, (31) 1.110 mg/mL.

A standard solution containing the 31 components was then diluted with methanol to obtain a series of standard working solutions that were used to establish calibration curves. All of the solutions were stored at 4 °C and then filtered through 0.22 μm membranes before LC-MS analysis.

2.5. UPLC-Q-Exactive-Orbitrap-MS conditions for qualitative analysis

In this study, a UPLC system tandem Q-Exactive/MS spectrometer (Thermo Fisher Scientific, CA, USA) equipped with a heated electrospray ionization (HESI) probe was employed. Halo C_{18} (2.1 mm × 100 mm, 2.7 μm, Advanced materials technology, DE, USA) column was used with the flow rate being set as 0.3 mL/min and column temperature was maintained at 35 °C. The binary solvent system consisted of 0.05 % aqueous formic acid (A) and 0.05 % formic acid in acetonitrile (B) and the linear gradients was as follows: 5 % B at 0–2 min; 5–95 % B at 2–24 min; 95 % B at 42–47 min. And an aliquot of 5 μL was injected.

Detection was performed using a Q-Exactive™ hybrid quadrupole-Orbitrap mass spectrometer in both positive and negative ionization modes. The optimal analysis conditions were set as follows: ion source, heated electrospray ionization probe; capillary temperature: 350 °C; spray voltage: 3.0 KV; sheath gas: 45 Arb; auxiliary gas: 10 Arb; mass collecting range: *m/z* 100-1500. The full scan and fragment spectra were collected at the resolutions of 70,000 and 17,500, respectively. The collision energy was 30 eV, 50 eV and 70 eV. The possible elemental composition was obtained through Xcalibur 3.0 (Thermo Fisher Scientific, CA, USA), and only those formulas with an error less than 10 ppm were accepted.

2.6. UPLC-QQQ-MS/MS conditions for quantitative

Chromatographic analysis was performed in an UltiMate 3000 UHPLC system (Thermo Fisher Scientific, CA USA). An Agilent ZORBAX SB-C_{18} column (250 mm × 4.6 mm, 5 μm, Agilent Technologies, CA, USA) was employed and the column temperature was maintained at 40 °C. The mobile phase consisted of 0.1 % aqueous formic acid (A) and acetonitrile (B), using a gradient elution of 8 % B at 0–10 min; 8–10 % B at 10–25 min; 10–15 % B at 25–27 min; 15 % B at 27–45 min; 15–30 % B at 45–65 min; 30–99 % B at 65–70 min; 99-8% B at 70–75 min; 8 % B at 75–80 min. The flow rate was 1 mL/min, with an injection volume of 5 μL.

A Thermo TSQ QUANTIS triple quadrupole mass spectrometer connected with an ESI interface was employed. To gain more information on the structural identification, each sample was analyzed in negative ion modes. The multiple reaction monitoring (MRM) conditions were optimized by infusion of the reference standard. The parameters in the source were set as follows: capillary voltage of 3.0 KV; sheath gas of 50 Arb; aux gas of 20 Arb; ion transfer tube temperature of 370 °C; and vaporizer temperature of 358 °C.

2.7. Quantitative method validation

The developed UPLC-QQQ-MS/MS method was validated by determining calibration curves, limits of detections (LODs), limits of quantifications (LOQs), precision, repeatability, stability and recovery. The standard solution containing the 31 components was diluted with methanol in 6 different multiples, and then the solution was analyzed using the UPLC-QQQ-MS/MS chromatographic Conditions. The calibration curve was established by plotting the peak area (Y) versus the corresponding concentration (X). The detection limit was the concentration of standard solution with a signal-to-noise ratio (S/N) of 3 (LOD), and the quantitative limit was the concentration of standard of standard solution with an S/N of 10 (LOQ). The analysis of the intra- and inter-day precisions of the method was evaluated with 6 replicate injections within one day (n = 6), and additionally on 3 consecutive days (n = 3), respectively.

The repeatability was determined with six solutions prepared in parallel from the same sample, and the RSDs of 31 compounds were calculated. For investigating the stability, the same sample solution was injected at 0, 2, 4, 8, 12 and 24 h, respectively. A recovery test was used to evaluate the accuracy of the developed method. Precise amounts of reference compound were added to 0.25 g of sample powder, which was then extracted and analyzed as described above. The recoveries was calculated according to the following formula: Recovery (%) = (Measured value of spiked sample-Measured value of the sample)/(Amount spiked) × 100 %.

2.8. Statistical data analysis

In order to obtain a good overview of the sample classification from the different medical parts of *L. japonica*, the contents of 31 components in all samples were used to perform PCA and OPLS-DA analysis with the SIMCA v14.1 software (Umetrics AB, Umea, Sweden). The hierarchical clustering analysis heat map was drawn using the Origin 2021 (OriginLab, Northampton, MA, USA). In
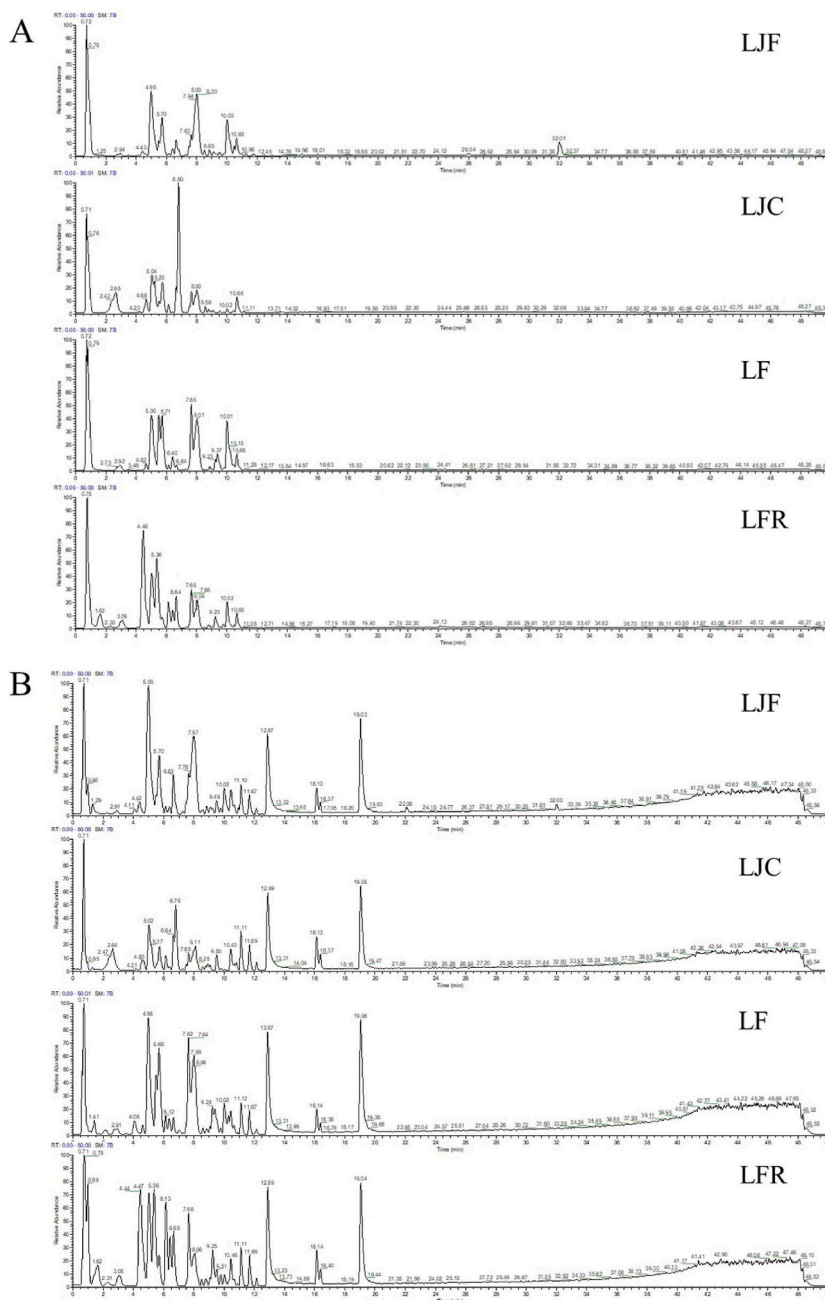

Fig. 1. The BPC of LJF, LJC, LF and LFR: negative mode (A), positive mode (B).

addition, all the experimental data were statistically compared by one-way ANOVA (SPSS 16.0 for Windows, IBM, Armonk, NY, USA). The GraphPad Prism 9.0 software (Graphpad Software, San Diego, CA, USA) was used to visualize the data.

3. Results

3.1. Identity and confirmation of the components in LJF, LJC, LF and LFR

The base peak chromatograms (BPC) of four herbal materials were obtained as shown in Fig. 1. The details for the identified compounds, such as the retention time (t_R), the molecular formula, the experimental molecular mass and MS^2 (fragment ion) information, are summarized in Table 1. All the compounds in the four herbal materials were identified based on data reported in the literature or according to proposed MS fragmentation. The mass error for molecular ions in all identified compounds was within 10 ppm, indicating that the experimental molecular formula well-matched with the quasimolecular ions, theoretical molecular ions and fragment ions.

A total of 86 compounds were detected and tentatively identified, including 25 flavonoids, 24 phenolic acids, 24 iridoid glycosides, 9 amino acids, and 4 other components. There are 54 common components among the four medical parts. Among them, LJF contains the most abundant components (80 kinds), LFR was the second, and (73 components), LF was the third (68 components), and LJC contains the least kinds of ingredients (62 components). Chrysoeriol, tricin 7-O-glucoside, kingiside, lonijaposide N, lonijaposide T, lonijapospiroside A, loniphenyruviridoside B and threonine were detected only in LJF, while they were not detected in other medical parts. 7-Epi-loganin was detected only in LJC, mauritianin was detected only in LF, and 2 components including cyanidin 3,5-diglucoside and peonidin-3,5-O-diglucoside were only detected in LFR. The above research results indicated that herbal medicines from different parts of *L. japonica* have similar chemical compositions, but differ in specific component compositions.

3.1.1. The fragmentation regularity of flavonoids

Flavonoids are the main active ingredients in *L. japonica*. A total of 42 flavonoids were identified in this study, including dihydroflavone, flavonol, flavone and anthocyanin. As far as flavonoid glycosides are concerned, the molecular ion peak intensity is generally weak, and the base peak is usually the fragment peak of aglycon [45].

A total of 21 flavones and flavonols were identified, including compounds **51**, **53**, **55**, **56**, **57**, **58**, **60**, **62**, **64**, **65**, **66**, **67**, **68**, **70**, **76**, **78**, **81**, **82**, **83**, **84**, **85**. Flavones and flavonols are prone to Retro-Diels-Alder (RDA) cracking, and generally produce fragment ions such as $[^{1,3}A]^-$ and $[^{1,4}A]^-$. The cracking mode is shown in Fig. A.3. And then the plasma fragments of H_2O, CO and CO_2 were lost. Compound **55** showed a [M−H]$^-$ ion at m/z 463.0890, and then the major fragment ions were observed at m/z 301.0345, 300.0279, 271.0251, 255.0298, 151.0025. It was speculated that m/z 301.0345 and 300.0279 were formed by the loss of one molecule of galactose by [M−H]$^-$ in the secondary mass spectrometry. Then the further loss of [−CH_2O] and [−C_2H_2O] to make acquired of ions at m/z 271.0251 and 255.0298, respectively. After the compound lost galactose, 151.0025 $[^{1,3}A]^-$ fragment ions were cleaved by RDA. By analyzing the cleavage process, compound **55** was preliminarily identified as hyperoside [39], Fig. A.4A. Compound **83** showed a [M−H]$^-$ ion at m/z 299.0570. In its MS/MS spectra, the [M−H−CH_3]$^-$ ion at m/z 284.0333, the [M−H−CH_3−CO]$^-$ ion at m/z 256.0375 and the $[^{1,3}A]^-$ ion at m/z 151.0042. Therefore, compound **83** was deduced to be diosmetin [40].

Compounds **50** and **86** were identified as dihydroflavones. Dihydroflavonoids are prone to have RDA reactions, where 1, 3 bonds of C ring are more likely to break to produce $[^{1,3}A]^-$ and $[^{1,3}B]^-$ [45]. Compound **50** exhibited the precursor ion [M − H]$^-$ at m/z 449.1083 in the negative mode. The ion at m/z 287.0566 was obtained by the loss of glucose from the precursor ion at m/z 449.1083, while the ion at m/z 269.0462 was produced by continuous loss of H_2O. Other fragments are produced by C ring cleavage, such as 151.0024 $[^{1,3}A]^-$; 135.0438 $[^{1,3}B]^-$. Therefore, compound **50** was deduced to be eriodictyol-7-O-glucoside [36], Fig. A.4B.

In addition to the above types of compounds, anthocyanins were identified in LFR, including compound **16** and compound **24**. In the natural state, anthocyanins often bind to various monosaccharides in plants and form glycosides, which named anthocyanins. Anthocyanins are positively charged, so they are often detected in the form of [M]$^+$. In the positive ion mode, compound **16** exhibited a molecular ion [M]$^+$ at m/z 611.1596. The fragment ion at m/z 449.1074 was formed by losing a molecule of glucose on the excimer ion [M]$^+$. The fragment ion continued to lose a molecule of glucose to form m/z 287.0541 [30]. The molecular ion peak [M]$^+$ of compound **24** was at m/z 625.1753. After the molecular ion peak lost two molecules of glucose, a fragment ion at m/z 301.0698 [M−2Glc]$^+$ was generated. Since the peony anthocyanin aglycone had an OCH_3 structure, a fragment ion at m/z 286.0463 [M−Glc−CH_3]$^+$ was generated after further loss of CH_3 fragment [33].

3.1.2. The fragmentation regularity of phenolic acids

The structural skeletons of phenolic acids were mostly C6–C1 types (such as quinic acid and protocatechuic acid) and C6–C3 types (such as caffeic acid and ferulic acid). Each phenolic acid component can be condensed to form a component with an ester structure (such as chlorogenic acid and 3-O-feruloylquinic acid) [46], the phenolic acids in different parts of *L. japonica* are mostly composed of one or more caffeic acid substituents bound to a portion of quinic acid. The MS/MS spectra usually have a basic peak at [M−H−CA]$^-$, and then lost H_2O, CO_2, or CO, which usually produce various ions, such as 353 [M−H−CA]$^-$, 335 [M−H−CA−H_2O]$^-$, 179, 135, 127, and so forth [29]. Therefore, compounds **4**, **28** and **52** were taken as examples to clarify the cleavage rules of phenolic acids in different parts of *L. japonica* (CA represented caffeic acid and QA represented quinic acid).

Compound **4** was speculated that its molecular formula might be $C_7H_{12}O_6$ based on the ion [M−H]$^-$ at m/z 191.0554. Compound **4** produced the fragment ions at m/z 173.0084 [M−H−H_2O]$^-$, 127.0389 [M−H−2H_2O−CO]$^-$, 111.0073 [M−H−2H_2O−CO_2]$^-$ and 85.0279 [M−H−2H_2O−CO−C_2H_2O]$^-$. Based on the above mass spectrometry data and references, the compound was identified as

Table 1
Identification of components in different parts of *L. japonica* (flowers, stems, leaves and fruits)

No.	t_R /min	Molecular formula	MS^1(m/z)	Error/ ppm	MS^2(m/z)	Identification	Aerial parts				References
							LJF	LJC	LF	LFR	
1	0.62	$C_6H_{14}N_4O_2$	175.1187 [M+H]$^+$	−1.14	158.0923[M+H−NH$_3$]$^+$; 130.0974[M+H−NH$_3$−CO]$^+$; 116.0708	Arginine	+	+	−	+	[26]
2	0.66	$C_{12}H_{22}O_{11}$	341.1096 [M−H]$^-$	5.28	179.0553; 161.0445[M−H−Glc]$^-$; 89.0229	Sucrose	+	+	+	+	[27]
3	0.69	$C_6H_{14}N_2O_2$	147.1123 [M+H]$^+$	−3.40	130.0498[M+H−NH$_3$]$^+$; 84.0449[M+H−HCOOH−NH$_3$]$^+$	Lysine	+	+	+	+	−
4	0.72	$C_7H_{12}O_6$	191.0554 [M−H]$^-$	2.09	173.0084[M−H−H$_2$O]; 127.0389[M−H−2H$_2$O−CO]$^-$; 154.9974; 111.0073 [M−H−2H$_2$O−CO$_2$]$^-$; 85.0279[M−H−2H$_2$O−CO−C$_2$H$_2$O]$^-$	Quinic acid	+	+	+	+	[28]
5	0.74	$C_6H_{12}O_6$	179.0552 [M−H]$^-$	1.12	161.0446; 87.0072; 71.0123	Fructose	+	+	+	+	[27]
6	0.76	$C_4H_9NO_3$	120.0654 [M+H]$^+$	−0.83	103.0589[M+H−NH$_3$]$^+$; 84.0450; 74.0607[M+H−HCOOH]$^+$	Threonine	+	−	−	−	−
7	0.86	$C_5H_9NO_2$	116.0707 [M+H]$^+$	0.86	70.0658[M+H−HCOOH]$^+$	Proline	+	−	−	+	[29]
8	0.91	$C_6H_{13}NO_2$	132.1017 [M+H]$^+$	−1.51	86.0969[M+H−HCOOH]$^+$; 69.0706[M+H−HCOOH−NH$_3$]$^+$	Isoleucine	+	−	+	+	[29]
9	0.92	$C_9H_{11}NO_3$	182.0814 [M+H]$^+$	1.10	165.0545[M+H−NH$_3$]$^+$; 147.0438[M+H−NH$_3$−H$_2$O]$^+$; 136.0756 [M+H−HCOOH]$^+$; 123.0441; 119.0492; 91.0545	Tyrosine	+	+	+	−	[26]
10	1	$C_5H_5N_5O$	152.0565 [M+H]$^+$	−1.32	135.0300[M+H−NH$_3$]$^+$; 110.0351	Guanine	+	+	+	+	−
11	1.4	$C_9H_{11}NO_2$	166.0855 [M+H]$^+$	−4.82	149.0593[M+H−NH$_3$]$^+$; 120.0808[M+H−HCOOH]$^+$; 103.0545 [M+H−HCOOH−NH$_3$]$^+$	Phenylalanine	+	+	+	+	[29]
12	2.08	$C_7H_6O_4$	153.0182 [M−H]$^-$	0.00	135.0076[M−H−H$_2$O]$^-$; 109.0451[M−H−CO$_2$]$^-$; 91.0174 [M−H−CO$_2$−H$_2$O]$^-$	Protocatechuic acid	+	+	+	+	[29]
13	2.3	$C_5H_{11}NO_2$	118.0863 [M+H]$^+$	0.00	72.0814[M+H−HCOOH]$^+$; 58.0659[M+H−C$_2$H$_4$O$_2$]$^+$	Valine	+	+	+	+	[29]
14	2.3	$C_{11}H_{12}N_2O_2$	205.0979 [M+H]$^+$	3.41	188.0702[M+H−NH$_3$]$^+$; 170.0598[M+H−NH$_3$−H$_2$O]$^+$; 159.0914 [M+H−HCOOH]$^+$; 146.0598	Tryptophan	−	+	+	+	−
15	2.47	$C_{16}H_{18}O_9$	353.0884 [M−H]$^-$	4.81	191.0554[M−H−CA]$^-$; 179.0342[CA−H]$^-$; 135.0440[CA−H−H$_2$O−C$_2$H$_2$]$^-$	1-O-caffeoylquinic acid	+	+	+	+	[29]
16	2.65	$C_{27}H_{31}O_{16}^+$	611.1596[M]$^+$	−1.80	449.1074[M−Glc]$^+$; 287.0541[M−2Glc]$^+$	Cyanidin 3,5-diglucoside	−	−	−	+	[30]
17	2.91	$C_{16}H_{18}O_9$	353.0882 [M−H]$^-$	4.25	191.0554[M−H−CA]$^-$; 179.0341[CA−H]$^-$; 135.0439 [CA−H−H$_2$O−C$_2$H$_2$]$^-$	Neochlorogenic acid	+	+	+	+	[28]
18	3.52	$C_7H_6O_3$	137.0232 [M−H]$^-$	-0.73	119.0126[M−H−H$_2$O]$^-$; 93.0331[M−H−CO$_2$]$^-$	4-Hydroxybenzoic acid	+	+	+	+	−
19	3.61	$C_{16}H_{24}O_{10}$	375.1295 [M−H]$^-$	2.40	213.0765[M−H−Glc]$^-$; 169.0861[M−H−Glc−CO$_2$]$^-$; 151.0754 [M−H−Glc−CO$_2$−H$_2$O]$^-$	8-Epi-loganin acid	+	+	+	+	[29]
20	4.11	$C_{16}H_{22}O_{10}$	373.1138 [M−H]$^-$	2.41	193.0499[M−H−Glc−H$_2$O]$^-$; 149.0596 [M−H−Glc−H$_2$O−CO$_2$]$^-$; 101.0229	Swertiamarin	+	+	+	+	[29]
21	4.48	$C_{17}H_{24}O_{10}$	387.1283 [M−H]$^-$	−0.77	341.1098 [M−H−H$_2$O−CO]$^-$; 179.0707 [M−H−Glc−H$_2$O−CO]$^-$; 119.0337	Secologanin	+	+	+	+	[31]
22	4.55	$C_{25}H_{24}O_{12}$	515.1207 [M−H]$^-$	4.46	191.0553[M−H−2CA]$^-$; 135.0439[CA−H−CO]$^-$	1,3-O-Dicaffeoylquinic acid	+	+	+	+	[32]
23	4.64	$C_{16}H_{24}O_{10}$	375.1299 [M−H]$^-$	3.47	213.0764[M−H−Glc]$^-$; 169.0860[M−H−Glc−CO$_2$]$^-$; 151.0753 [M−H−Glc−CO$_2$−H$_2$O]$^-$	Loganic acid	+	+	+	+	[29]
24	4.69	$C_{28}H_{33}O_{16}^+$	625.1753[M]$^+$	−1.60	301.0698[M−2Glc]$^+$; 286.0463[M−2Glc−CH$_3$]$^+$	Peonidin-3,5-O-diglucoside	−	−	−	+	[33]

(continued on next page)

Table 1 (*continued*)

No.	t_R /min	Molecular formula	MS^1(m/z)	Error/ ppm	MS^2(m/z)	Identification	Aerial parts				References
							LJF	LJC	LF	LFR	
25	4.74	$C_{17}H_{26}O_{11}$	451.1468 [M+HCOO]$^-$	4.88	243.0873[M−H−Glc]$^-$; 225.0764[M−H−Glc−H$_2$O]$^-$; 179.0550	Morroniside	+	+	+	+	[29]
26	5.1	$C_{16}H_{18}O_9$	353.0869 [M−H]$^-$	0.57	191.0554[M−H−CA]$^-$; 179.0341[CA−H]$^-$; 135.0439 [CA−H−H$_2$O−C$_2$H$_2$]$^-$	Chlorogenic acid	+	+	+	+	[28]
27	5.21	$C_{26}H_{33}NO_{11}$	536.2112 [M+H]$^+$	−2.61	304.1172; 218.0807	Lonijaposide N	+	−	−	−	−
28	5.51	$C_9H_8O_4$	179.0340 [M−H]$^-$	0.56	135.0439[M−H−CO$_2$]$^-$; 107.0490[M−H−C$_2$O$_3$]$^-$; 89.0230 [M−H−C$_2$O$_3$−H$_2$O]$^-$	Caffeic acid	+	+	+	+	[28, 29]
29	5.57	$C_{16}H_{22}O_{11}$	389.1083 [M−H]$^-$	1.28	345.1219[M−H−CO$_2$]$^-$; 209.0460[M−H−Glc−H$_2$O]$^-$; 183.0652 [M−H−Glc−CO$_2$]$^-$; 165.0547[M−H−Glc−CO$_2$−H$_2$O]$^-$; 121.0645	secologanoside	+	+	+	+	[34]
30	5.73	$C_{27}H_{33}NO_{13}$	580.2014 [M+H]$^+$	−1.72	348.1071; 202.0496	Lonijaposide A or H	+	−	−	+	−
31	5.78	$C_{16}H_{22}O_{10}$	373.1148 [M−H]$^-$	5.09	193.0503[M−H−Glc−H$_2$O]$^-$; 149.0598[M−H−Glc−H$_2$O−H$_2$O−C$_2$H$_2$]$^-$; 141.0183; 101.0230	Secologanic acid	+	+	+	+	[29]
32	6.04	$C_{17}H_{26}O_{10}$	435.1495 [M+HCOO]$^-$	−0.46	227.0922[M−H−Glc]$^-$; 209.0812[M−H−Glc−H$_2$O]$^-$; 127.0388; 101.0229	7-Epi-loganin	−	+	−	−	[31]
33	6.13	$C_{21}H_{32}O_{14}$	507.1721 [M−H]$^-$	2.56	357.1198; 327.1090; 283.1206; 196.0667	Secologanoside A	+	+	+	+	[27]
34	6.35	$C_{16}H_{18}O_9$	353.0869 [M−H]$^-$	0.57	191.0554[M−H−CA]$^-$; 179.0341[CA−H]$^-$	Cryptochlorogenic acid	+	+	+	+	[28]
35	6.42	$C_{18}H_{26}O_{10}$	447.1499 [M+HCOO]$^-$	0.45	401.1452[M−H]$^-$; 269.1035	7-O-Ethyl sweroside	+	+	+	+	[35]
36	6.54	$C_{16}H_{18}O_8$	337.0938 [M−H]$^-$	6.23	191.0554[QA−H]$^-$; 163.0390[M−H−QA]$^-$; 93.0331	3-p-Coumaroylquinic acid	+	+	+	+	[35]
37	6.58	$C_6H_6O_3$	127.039 [M+H]$^+$	0.00	127.0390; 99.0444[M−H−CO]$^+$; 69.0342	Phloroglucinol	+	+	+	+	−
38	6.71	$C_{28}H_{35}NO_{13}$	594.2172 [M+H]$^+$	−1.51	432.1653; 362.1226; 216.0651	Lonijaposide T	+	−	−	−	−
39	6.72	$C_{16}H_{22}O_9$	403.1234 [M+HCOO]$^-$	−0.25	195.0656[M−H−Glc]$^-$; 151.0753[M−H−Glc−CO$_2$]$^-$; 125.0230 [M−H−Glc−CO$_2$−C$_2$H$_2$]$^-$	Sweroside	+	+	+	+	[31]
40	6.72	$C_{17}H_{24}O_{10}$	433.1358 [M+HCOO]$^-$	4.16	387.2008[M−H]$^-$; 225.0767[M−H−Glc]$^-$; 179.0546	7-Epi-Vogeloside	+	+	+	+	[14]
41	6.75	$C_{17}H_{26}O_{10}$	435.1493 [M+HCOO]$^-$	−0.92	227.0923[M−H−Glc]$^-$; 209.0821[M−H−Glc−H$_2$O]$^-$; 127.0388; 101.0229	Loganin	+	+	+	+	[31]
42	7.25	$C_{17}H_{24}O_{11}$	403.1242 [M−H]$^-$	1.74	241.1541[M−H−Glc]$^-$; 191.0555; 173.0446; 134.0360	Kingiside	+	−	−	−	[29]
43	7.35	$C_{17}H_{20}O_9$	367.1041 [M−H]$^-$	4.63	191.05544[QA−H]$^-$; 173.0447[QA−H−H$_2$O]$^-$; 127.0387 [QA−H−2H$_2$O−CO]$^-$; 111.0435	3-O-Feruloylquinic	+	+	+	+	[29]
44	7.54	$C_{27}H_{33}NO_{13}$	580.2018 [M+H]$^+$	−1.03	348.1071; 260.0910	Lonijaposide A or H	+	−	−	+	−
45	7.6	$C_{10}H_{10}O_4$	193.0501 [M−H]$^-$	3.11	178.0257[M−H−CH$_3$]$^-$; 161.0259[M−H−OCH$_3$]$^-$; 133.0282 [M−H−CO$_2$−CH$_3$]$^-$	Ferulic acid	−	+	−	+	[29]
46	7.73	$C_{17}H_{24}O_{11}$	403.1212 [M−H]$^-$	−5.71	371.0972; 223.0615[M−H−Glc−H$_2$O]$^-$; 165.0546; 149.0230 [M−H−Glc−H$_2$O−CH$_3$OH−C$_2$H$_2$O]$^-$; 121.0282 [M−H−Glc−H$_2$O−CH$_3$OH−C$_2$H$_2$O−CO]$^-$	Secoxyloganin	+	+	+	+	[29]
47	7.96	$C_{10}H_{10}O_4$	195.0643 [M+H]$^+$	−4.61	177.0545[M+H−H$_2$O]$^+$; 151.0388; 109.0286	Caffeic acid methyl ester	+	+	+	−	[32]

Table 1 (*continued*)

No.	t_R /min	Molecular formula	MS^1(m/z)	Error/ ppm	MS^2(m/z)	Identification	Aerial parts				References
							LJF	LJC	LF	LFR	
48	8.11	$C_{17}H_{20}O_9$	367.1041 [M−H]$^-$	4.63	191.0554[M−H−CA]$^-$; 173.0445 [M−H−CA−CH$_3$OH]$^-$; 134.0361; 127.0388[M−H−CA−CH$_3$OH−H$_2$O−CO]$^-$; 93.0331	3-O-Caffeoylquinic acid methyl ester	+	+	+	+	[29]
49	8.2	$C_{25}H_{31}NO_{12}$	538.1907 [M+H]$^+$	−2.23	376.1754; 358.1640	Lonijaposide B	+	+	+	+	−
50	8.84	$C_{21}H_{22}O_{11}$	449.1083 [M−H]$^-$	4.68	287.0564[M−H−Glc]$^-$; 269.0464[M−H−Glc−H$_2$O]$^-$; 151.0024; 135.0438	Eriodictyol-7-O-glucoside	+	+	+	+	[36]
51	8.86	$C_{27}H_{30}O_{16}$	609.1471 [M−H]$^-$	3.45	301.0346[M−H−Rha−Glc]$^-$; 271.0251[M−H−Rha−Glc−CO]$^-$;255.0299; 243.0298; 178.9976; 151.0026; 107.0124	Rutin	+	+	+	+	[28]
52	8.94	$C_{34}H_{30}O_{15}$	677.1545 [M−H]$^-$	6.50	515.1198[M−H−CA]$^-$; 353.0884[M−H−2CA]$^-$; 179.0341[CA−H]$^-$; 191.0555[QA−H]$^-$;135.0439 [CA−H−H$_2$O−C$_2$H$_2$]$^-$	3,4,5-Tricaffeoylquinic acid	+	+	+	+	[29]
53	9	$C_{33}H_{40}O_{19}$	741.2222 [M+H]$^+$	−2.02	287.0543; 153.0180; 85.0289	Mauritianin	−	−	+	−	[37]
54	9.01	$C_{28}H_{34}N_2O_{11}$	575.2231 [M+H]$^+$	2.44	413.1714[M+H−Glc]$^+$; 396.1427; 188.0703	5 (S) -5-carboxystrictosidine	+	+	+	+	[38]
55	9.06	$C_{21}H_{20}O_{12}$	463.0890 [M−H]$^-$	4.10	301.0345[M−H−Gal]$^-$; 300.0279; 271.0251[M−H−Gal−CH$_2$O]$^-$; 255.0298[M−H−Gal−CH$_2$O−C$_2$H$_2$O]$^-$; 243.0298 [M−H−Gal−CH$_2$O−CO]$^-$; 151.0025; 107.0123	Hyperoside	+	+	+	+	[39]
56	9.17	$C_{21}H_{20}O_{12}$	465.1062 [M+H]$^+$	7.31	303.0493[M+H−Glc]$^+$	Isoquercitrin	+	+	+	+	−
57	9.32	$C_{27}H_{30}O_{15}$	593.1523 [M−H]$^-$	3.71	447.0945[M−H−Rha]$^-$; 285.0405[M−H−Rha−Glc]$^-$; 284.0330	Lonicerin	+	+	+	+	[29]
58	9.33	$C_{21}H_{20}O_{11}$	447.0941 [M−H]$^-$	4.25	285.0405[M−H−Glc]$^-$; 284.0331; 267.0297[M−H−Glc−H$_2$O]$^-$; 255.0298; 133.0282	Luteoloside	+	+	+	+	[29]
59	9.64	$C_9H_8O_3$	165.0544 [M+H]$^+$	−1.21	147.0439[M+H−H$_2$O]$^+$; 91.0547	4-Hydroxycinnamic acid	+	−	+	+	[32]
60	9.86	$C_{27}H_{30}O_{15}$	593.1522 [M−H]$^-$	3.54	285.0405[M−H−Rha−Glc]$^-$; 255.0300; 227.0347	Kaempferol-3-O-rutinoside	+	+	+	+	[29]
61	9.96	$C_{25}H_{24}O_{12}$	517.1336 [M+H]$^+$	−0.77	163.0387; 145.0283; 117.0336	Isochlorogenic acid B	+	+	+	+	[32]
62	10	$C_{21}H_{20}O_{11}$	447.0939 [M−H]$^-$	3.80	285.0396[M−H−Glc]$^-$; 284.0331; 255.0300; 227.0347	Astragalin	+	+	+	+	[29]
63	10.01	$C_{25}H_{24}O_{12}$	515.1197 [M−H]$^-$	2.52	353.0882[M−H−CA]$^-$; 335.0786[M−H−CA−H$_2$O]$^-$; 191.0555 [M−H−2CA]$^-$; 179.0341; 173.0448[M−H−2CA−H$_2$O]$^-$; 161.0233; 135.0439[CA−H−CO]$^-$	Isochlorogenic acid A	+	+	+	+	[29]
64	10.02	$C_{28}H_{32}O_{16}$	623.1599 [M−H]$^-$	−1.28	315.0511[M−H−Rha−Glc]$^-$; 299.0200; 271.0252[M−H−CO$_2$]$^-$; 243.0296	Narcissin	+	−	+	+	−
65	10.24	$C_{22}H_{22}O_{12}$	477.1045 [M−H]$^-$	3.56	314.0439[M−H−Glc]$^-$; 285.0410[M−H−Glc−C$_2$H$_5$]$^-$; 271.0250; 243.0297	Isorhamnetin-3-O-glucoside	+	−	+	+	[40]
66	10.25	$C_{27}H_{30}O_{14}$	577.1570 [M−H]$^-$	3.12	413.0889[M−H−Rha−H$_2$O]$^-$, 269.0457 [M−H−Rha−Glc]$^-$	Rhoifolin	+	+	+	+	[29]
67	10.26	$C_{21}H_{20}O_{10}$	431.0989 [M−H]$^-$	3.94	269.0442; 268.0381[M−H−Glc]$^-$; 240.0423[M−H−Glc−H$_2$O]$^-$; 239.0347	Apigenin 7-glucoside	+	+	+	+	[40]
68	10.27	$C_{16}H_{12}O_7$	317.0649 [M+H]$^+$	−2.21	302.0414; 274.0469; 229.0490; 153.0181	Isorhamnetin	+	−	−	+	[41]
69	10.58	$C_{34}H_{46}O_{19}$	757.2573 [M−H]$^-$	3.00	595.2079[M−H−Glc]$^-$; 525.1618 [M−H−Glc−H$_2$O−C$_3$O]−	(E)-Aldosecologanin	+	+	+	+	[39]
70	10.63	$C_{28}H_{32}O_{15}$	607.1666 [M−H]$^-$	1.48	299.0566[M−H−Rha−Glc]$^-$; 284.0329; 255.0299; 173.0446	Diosmin	+	+	+	+	[40]

Table 1 (*continued*)

No.	t_R /min	Molecular formula	MS^1(m/z)	Error/ ppm	MS^2(m/z)	Identification	Aerial parts				References
							LJF	LJC	LF	LFR	
71	10.73	$C_{25}H_{24}O_{12}$	515.1197 [M−H]$^-$	2.52	353.0882[M−H−CA]$^-$; 191.0553[M−H−2CA]$^-$; 179.0340; 173.0446; 161.0233; 135.0438[CA−H−CO]$^-$	Isochlorogenic acid C	+	+	+	+	[29]
72	10.94	$C_{34}H_{46}O_{19}$	757.2573 [M−H]$^-$	3.00	595.2079[M−H−Glc]$^-$; 525.1618 [M−H−Glc−H$_2$O−C$_3$O]$^-$	(Z)-Aldosecologanin	+	+	+	+	[39]
73	11.2	$C_{25}H_{24}O_{11}$	499.1266 [M−H]$^-$	6.21	353.0880[M−H−PA]$^-$; 191.0554; 179.0341; 163.0390; 135.0439; 119.0448	Coumaroyl caffeoylquinic acid	+	+	+	−	[29]
74	11.71	$C_{26}H_{26}O_{12}$	529.1363 [M−H]$^-$	4.16	353.0879[M−H−C$_{10}$H$_8$O$_3$]$^-$; 191.0554[M−H−C$_{10}$H$_8$O$_3$−CA]$^-$; 179.0341 [CA−H]$^-$; 173.0447; 155.0340	Feruloyl caffeoylquinic acid	+	−	+	+	[29]
75	11.8	$C_{27}H_{31}O_{12}N$	560.1785 [M+H]$^+$	4.11	328.0833; 284.0930	Lonijapospiroside A	+	−	−	−	−
76	12.1	$C_{15}H_{10}O_7$	303.0493 [M+H]$^+$	−1.98	257.0439[M+H−CO−H$_2$O]$^+$; 229.0492[M+H−CO−H$_2$O−CO]$^+$; 177.0543; 153.0179	Quercetin	+	−	+	+	[42]
77	12.23	$C_{26}H_{26}O_{12}$	529.1363 [M−H]$^-$	4.16	367.1038 [M−H−CA]$^-$,179.0340[CA−H]$^-$,161.0233 [CA−H−H$_2$O]$^-$,135.0439 [CA−H−CO$_2$]$^-$	4,5-O-Dicaffeoylquinic acid methyl ester	+	−	+	+	[29]
78	12.81	$C_{15}H_{10}O_6$	285.0409 [M−H]$^-$	5.26	241.0503[M−H−H$_2$O−C$_2$H$_2$]$^-$; 199.0394[M−H−H$_2$O−C$_4$H$_4$O]$^-$; 175.0391; 151.0025; 133.0282	Luteolin	+	+	+	+	[29]
79	12.91	$C_{11}H_{12}O_4$	207.0659 [M−H]$^-$	3.86	135.0439[M−H−C$_3$H$_4$O$_2$]$^-$; 133.0283	Ethyl caffeate	+	+	+	+	[43]
80	13.12	$C_{25}H_{28}O_{12}$	519.1470 [M−H]$^-$	−5.20	357.1563[M−H−Glc]$^-$; 173.0446	Loniphenyruviridoside B	+	−	−	−	−
81	14.42	$C_{23}H_{24}O_{12}$	491.1207 [M−H]$^-$	4.68	329.0671[M−H−Glc]$^-$; 314.0438; 299.0202; 271.0251	Tricin 7-O-glucoside	+	−	−	−	[29]
82	14.75	$C_{16}H_{12}O_6$	299.0570 [M−H]$^-$	6.69	284.0333[M−H−CH$_3$]$^-$; 256.0375[M−H−CH$_3$−CO]$^-$; 227.0352	Chrysoeriol	+	−	−	−	[29]
83	14.77	$C_{16}H_{12}O_6$	299.0570 [M−H]$^-$	6.69	284.0333[M−H−CH$_3$]$^-$; 256.0375[M−H−CH$_3$−CO]$^-$; 151.0042	Diosmetin	+	−	+	+	[40]
84	16.02	$C_{17}H_{14}O_7$	329.0676 [M−H]$^-$	6.38	229.1442; 211.1336	Tricin	+	+	+	+	[29]
85	16.7	$C_{15}H_{10}O_5$	269.0461 [M−H]$^-$	6.32	225.0551[M−H−CO$_2$]$^-$; 151.0024; 117.0331	Apigenin	+	−	+	+	[40]
86	18.78	$C_{15}H_{12}O_5$	273.0750 [M+H]$^+$	−2.93	153.0180; 119.0492	Naringenin	+	+	−	+	[44]

note: −, not detected; +, detected; QA, quinic acid; CA, caffeic acid

quinic acid [28].

Compound **28** showed a $[M-H]^-$ ion at m/z 179.0340, in its MS/MS spectra, the $[M-H-CO_2]^-$ ion at m/z 135.0453, the $[M-H-CO_2-CO]^-$ ion at m/z 107.0490 and the $[M-H-CO_2-CO-H_2O]^-$ ion at m/z 89.0230. According to these characteristic fragments and relevant literature, it is speculated to be caffeic acid [28].

Compound **52** was speculated that its molecular formula might be $C_{34}H_{30}O_{15}$ based on the ion $[M-H]^-$ at m/z 677.1524. Its MS^2 fragment had a loss of three caffeoyl moieties at m/z 515.1198 $[M-H-CA]^-$, 353.0884 $[M-H-2CA]^-$ and 191.0555 $[M-H-CA]^-$/ $[QA-H]^-$, which was consistent with the reported 3,4,5-tricaffeoylquinic acid [29]. The secondary mass spectrum and possible cleavage mode were shown in Fig. A.5.

3.1.3. The fragmentation regularity of iridoids

A total of 24 iridoid glycosides were identified in positive and negative ion modes, including common iridoid glycosides (compounds **19, 23, 25, 32, 41** and **42**), secoiridoid glycoside (compounds **20, 21, 29, 31, 33, 35, 39, 40, 46** and **80**), heterocyclic iridoid glycosides (compounds **27, 30, 38, 44, 49** and **75**) and polymeric iridoid glycosides (compounds **69** and **72**). These compounds existed in the form of glycosides, most of which were linked to a glucose at the C−1 position of the pyran ring. Due to the similarity of their structures, similar fragmentation rules were reflected in the mass spectrometry signals. The main cleavage pathway was as follows: ①The glycosidic bond at the C−1 position was broken, and the fragments generated due to the loss of glycosides; ②The carboxyl group at C−4 position and the hydroxyl groups at C−7 and C−8 fell off, and then the fragments was generated by losing CO_2 and H_2O; ③The fragments generated from the shedding of other substituents on the parent nucleus structure.

Taking compounds **23, 29** and **69** as examples, the mass spectrometry information analysis was carried out to explain the fragmentation rules of iridoid glycosides. Compound **23** was speculated that its molecular formula might be $C_{16}H_{24}O_{10}$ based on the ion $[M-H]^-$ at m/z 375.1299. The fragment ions at 213.0764, 169.0860 and 151.0753 were inferred to be caused by the loss of Glc, CO_2 and H_2O. Finally, it was verified that the compound **23** was loganic acid [29], Fig. A.6.

Compound **29** generated $[M-H]^-$ ion at m/z 389.1083, which was the base peak, and yielded characterized ions at m/z 345.1219 $[M-H-CO_2]^-$, m/z 183.0652 $[M-H-Glc-CO_2]^-$ and 165.0547 $[M-H-Glc-CO_2-H_2O]^-$. It was then tentatively characterized as secologanoside [34].

Compound **69** gave precursor ion $[M-H]^-$ at m/z 757.2573. Characteristic ion could be seen as at m/z 595.2079 after the loss of one molecule of glucose, and there were also some fragment ions unique to iridoid components, such as m/z 525.1618 $[M-H-Glc-H_2O-C_3O]^-$, which were consistent with the literature data of (E)-Aldosecologanin [39].

Table 2
The retention time, precursor ions, product ions, and multiple reaction monitoring (MRM) parameters of the 31 analytes in the negative ion mode.

| No.	compounds	Formula	Retention time	Precursor	Product	CE/eV	TB/V
1	Eriodictyol-7-O-glucoside	$C_{21}H_{22}O_{11}$	39.12	449.3	287.2	18	113
2	Hyperoside	$C_{21}H_{20}O_{12}$	39.81	463.3	300.2	28	143
3	Rutin	$C_{27}H_{30}O_{16}$	39.37	609.4	300.2	38	136
4	Isoquercitrin	$C_{21}H_{20}O_{12}$	41.65	463.3	300.2	28	138
5	Luteoloside	$C_{21}H_{20}O_{11}$	43.23	447.2	285.2	27	140
6	Lonicerin	$C_{27}H_{30}O_{15}$	46.30	593.4	285.1	43	163
7	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	50.56	593.4	285	32	142
8	Astragalin	$C_{21}H_{20}O_{11}$	52.30	447.3	255.1	40	123
9	Narcissin	$C_{28}H_{32}O_{16}$	52.84	623.4	315	31	137
10	Apigenin 7-glucoside	$C_{21}H_{20}O_{10}$	54.09	431.3	268.2	37	143
11	Rhoifolin	$C_{27}H_{30}O_{14}$	54.61	577.4	269.2	37	163
12	Luteolin	$C_{15}H_{10}O_6$	64.57	285.1	133.2	38	106
13	Apigenin	$C_{15}H_{10}O_5$	68.67	269.1	117.3	39	95
14	Diosmetin	$C_{16}H_{12}O_6$	69.02	299.1	284.2	23	105
15	Protocatechuic acid	$C_7H_6O_4$	7.16	153.1	109.3	16	71
16	Neochlorogenic acid	$C_{16}H_{18}O_9$	7.56	353.2	191.2	20	81
17	Chlorogenic acid	$C_{16}H_{18}O_9$	14.60	353.2	191.2	17	118
18	Cryptochlorogenic acid	$C_{16}H_{18}O_9$	16.57	353.2	173.2	17	130
19	Caffeic acid	$C_9H_8O_4$	18.20	179.1	135.2	16	73
20	Isochlorogenic acid B	$C_{25}H_{24}O_{12}$	50.07	515.3	353.3	21	125
21	Caffeic acid methyl ester	$C_{10}H_{10}O_4$	52.61	193.1	133.2	33	78
22	Isochlorogenic acid A	$C_{25}H_{24}O_{12}$	52.18	515.4	353.3	18	129
23	Isochlorogenic acid C	$C_{25}H_{24}O_{12}$	56.26	515.3	353.3	20	91
24	Loganic acid	$C_{16}H_{24}O_{10}$	10.29	373.2	213.2	18	120
25	Morroniside	$C_{17}H_{26}O_{11}$	13.47	451.3	243.2	18	79
26	Secologanic acid	$C_{16}H_{22}O_{10}$	17.78	373.2	193.2	16	123
27	Sweroside	$C_{16}H_{22}O_9$	26.53	403.3	125.2	22	84
28	Loganin	$C_{17}H_{26}O_{10}$	28.74	435.3	227.2	17	75
29	Secoxyloganin	$C_{17}H_{26}O_{11}$	31.87	403.3	121.2	27	158
30	(E)-Aldosecologanin	$C_{34}H_{46}O_{19}$	56.17	757.6	595.5	16	152
31	(Z)-Aldosecologanin	$C_{34}H_{46}O_{19}$	58.31	757.6	595.5	20	139
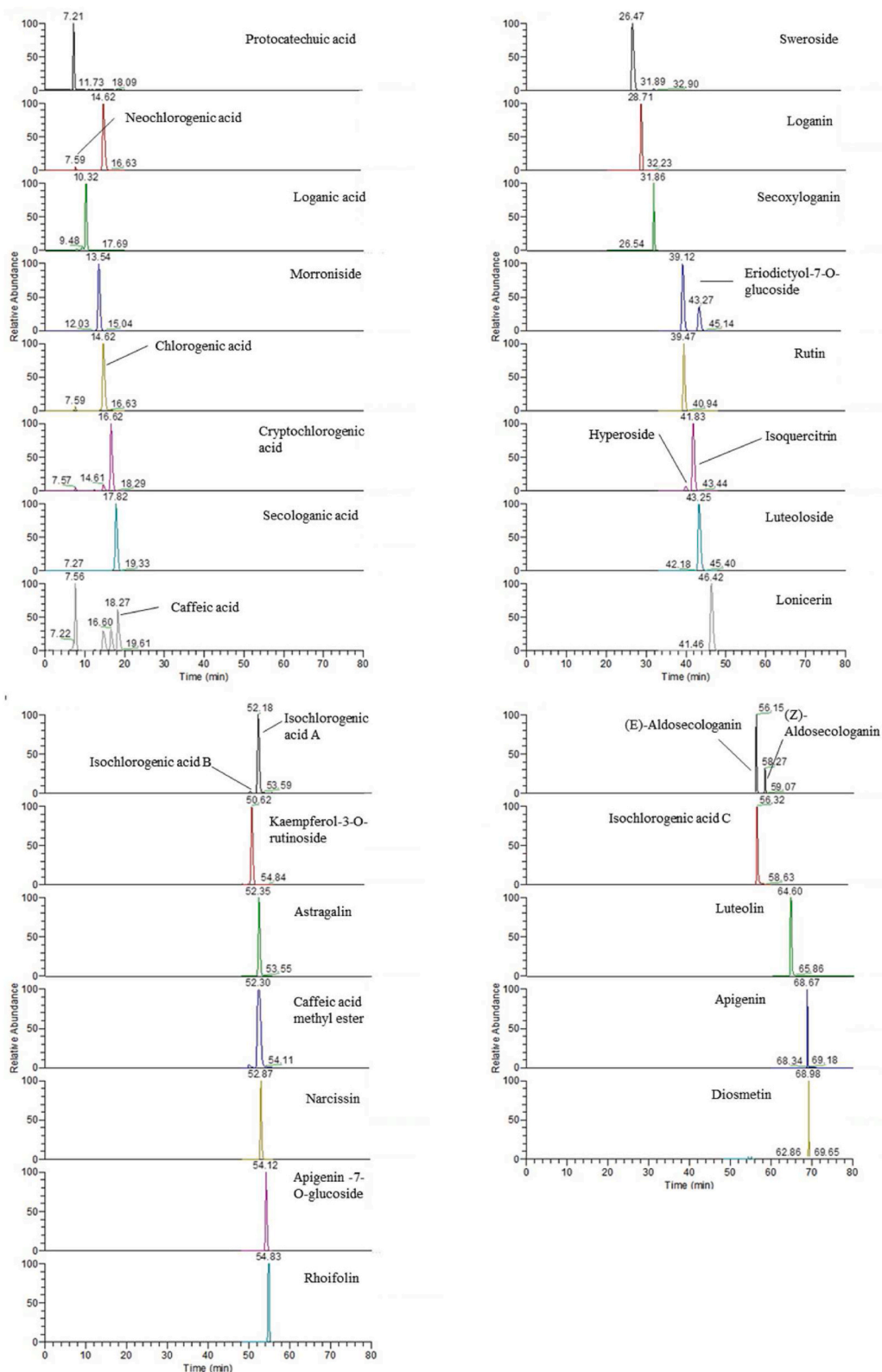
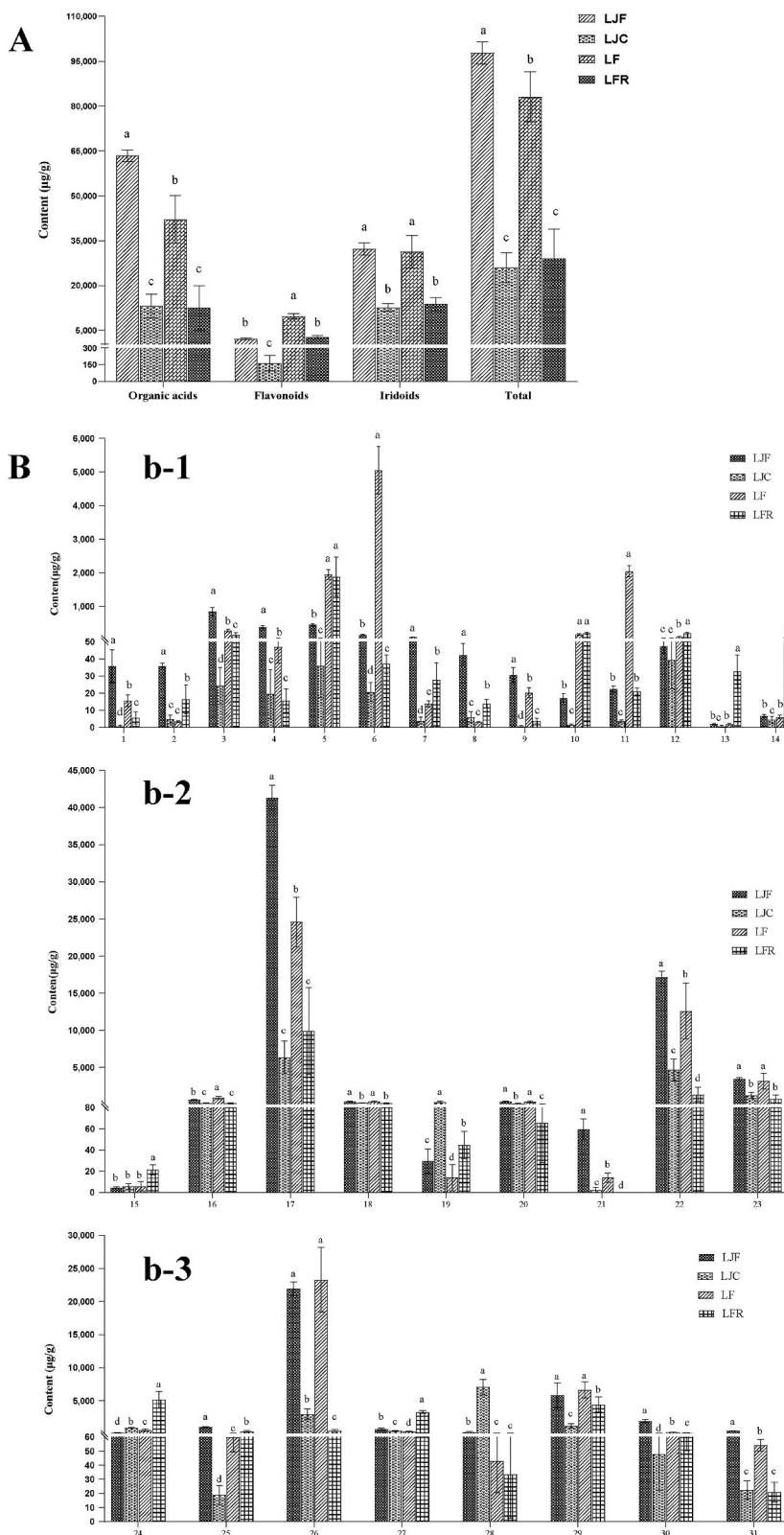**Fig. 2.** Multi-reaction monitoring (MRM) chromatograms of 31 reference standards.

Fig. 3. The content of 31 compounds in samples. (A) The content of organic acids, flavonoids and iridoids in different parts of *L. japonica*. (B) (b-1) flavonoids, (b-2) organic acids, (b-3) iridoids (X-axis 1-31 is the number of compounds, see Table 2 for compound names; Y-axis is the content (μg of compound/g)). Different lowercase letters indicate a significant difference, P < 0.05.

3.2. Quantification analysis

3.2.1. Optimization of extraction conditions

A total of 86 compounds were identified during qualitative analysis, however, commercially available controls of 31 compounds using quantitative analysis could be purchased, therefore, only 31 compounds were measured. In order to ensure that the 31 compounds in different parts of *L. japonica* demonstrate high extraction efficiency, the key factors, including the extraction solvent, solid-liquid ratio, and extraction time, were optimized. Firstly, a total of 0.5 g LJF was added to 20 mL of different proportions of methanol (30 % methanol, 50 % methanol, 75 % methanol, 90 % methanol) solutions and then subjected to ultrasonic extraction for 40 min at room temperature. The results showed that the total contents of 31 components were significantly higher in extraction solvent of 75 % methanol than with the extraction solvent of 30 % methanol, 50 % methanol and 90 % methanol ($P < 0.05$), so 75 % methanol was selected as the extraction solvent, Fig. A.7A.

Secondly, we optimized the solid-liquid ratio, and a total of 0.5 g LJF was added into 20 mL, 30 mL, 40 mL and 50 mL of 75 % methanol and subjected to ultrasonic extraction for 40 min at room temperature. The results showed that the solid-liquid ratio of 1:80 and 1:100 demonstrated the significantly higher total content of 31 components than that of 1:40 and 1:60, and there is no significant difference in solid-liquid ratio between 1:80 and 1:100 ($P > 0.05$). Considering the principle of saving solvent, the solid-liquid ratio is selected as 1:80, Fig. A.7B.

Finally, the extraction efficiencies of 20 min, 40 min, 60 min and 80 min were investigated. The results showed that the total contents of 31 components were significantly higher in extraction times of 60 min and 80 min than with the extraction times of 20 min and 40 min ($P < 0.05$). The content was no significant difference between 60 min and 80 min ($P > 0.05$), Fig. A.7C. In order to ensure the complete extraction and extraction efficiency, the extraction time was selected as 60 min. Thus, the optimal extraction condition was 75 % methanol as the extraction solvent, with a solid-liquid ratio of 1:80 g/mL, and ultrasonic extraction for 60 min.

3.2.2. Optimization of UPLC and MS conditions

To obtain good chromatographic behaviors, elution gradient program was optimized. Finally, the elution gradient was determined to be 0–10 min, 8 % B; 10–25 min, 8–10 % B; 25–27 min, 10–15 % B; 27–45 min, 15 % B; 45–65 min, 15–30 % B; 65–70 min, 30–99 % B; 70–75 min, 99-8% B; 75–80 min, 8 % B.

The mass spectrometry conditions of 31 compounds were optimized by three-way valve automatic injection mode, and a single standard solution of all standard compounds was injected into the ESI source. The full scan of 31 compounds was performed in positive and negative ion modes respectively. The results showed that the response values of 31 compounds were higher in negative ion mode, so negative ion mode scanning was selected. The ESI-QQQ-MS/MS parameter for analytes detection in multiple reaction monitoring (MRM) mode of compounds were optimized. The optimum collision energy (CE) and Tube Lens Voltage (TB) were selected according to each analyte (shown in Table 2). The MRM of the 31 constituents is shown in Fig. 2.

3.2.3. Validation of the quantitative analytical method

The regression equation, determination coefficient, linear range, limit of detection (LOD) and limit of quantitation (LOQ), precision, repeatability, stability, and recovery for the quantitative analysis of the 31 compounds are shown in Table A.1. The calibration curves for all 31 reference substances showed good linear regression (r > 0.9959) within the test ranges. The LODs of the 31 reference compounds were estimated to be 0.01–11.85 ng/mL, whereas the LOQs were 0.03–39.5 ng/mL. The RSD values of intra-day, inter-day, repeatability, stability of the 31 analytes ranged from 0.96 % to 4.51 %, 0.37 %–3.57 %, 1.37 %–5.47 % and 1.32 %–5.13 %, respectively. The mean recoveries varied between 95.11% and 101.44 %, with the RSD% less than 5.00 %, which verified the effectiveness of the proposed method.

3.2.4. Quantitative analysis of samples

The established UPLC-QQQ-MS/MS method was subsequently applied to the simultaneous determination of 31 bioactive constituents in 33 batches of samples, including 9 batches of LJF, 9 batches of LJC, 9 batches of LF and 6 batches of LFR. The results are shown in Table A.2. As shown in Fig. 3A, the content of 31 compounds in LJF was 97,809.67 µg/g, which was significantly higher than that of other parts ($P < 0.05$), followed by LF (83,111.1 µg/g), while there was no significant difference in the content of 31 compounds between LJC and LFR ($P > 0.05$). The total contents of phenolic acid of LJF (63,415.99 µg/g) were significantly higher than that of other medicinal parts ($P < 0.05$), followed by LF (42,160.51 µg/g), and there was no significant difference between LJC and LFR ($P > 0.05$). Total contents of flavonoids in LF were significantly higher than that in other medicinal parts ($P < 0.05$), which was 9696.46 µg/g, followed by LFR (2682.9 µg/g), and the content of total flavonoids in LJC (165.31 µg/g) was the lowest. Total contents of iridoid glycosides in LJF and LF were 32,230.72 and 31,254.13 µg/g, respectively, which were significantly higher than those in LJC and LFR ($P > 0.05$). There was no significant difference between LJC and LFR ($P > 0.05$).

The first five components with higher content in LJF were chlorogenic acid (41,286.56 µg/g), secologanic acid (21,931.32 µg/g), isochlorogenic acid A (17,152.41 µg/g), loganin (5830.28 µg/g) and isochlorogenic acid C (3436.13 µg/g). The first five components with higher content in LJC were loganin, isochlorogenic acid A, chlorogenic acid, secologanic acid and loganic acid, and their contents were 7057.73, 6358.52, 4696.80, 2971.08 and 1268.12 µg/g, respectively. The first five components with higher content in LF were chlorogenic acid, secologanic acid, isochlorogenic acid A, secoxyloganin and lonicerin, and their contents were 24,597.41, 23,281.28, 12,589.39, 6650.70 and 5051.02 µg/g, respectively. The top five components with higher content in LFR were chlorogenic acid, loganic acid, secoxyloganin, sweroside and luteoloside, and their contents were 9899.02, 5167.23, 4426.18, 3283.16 and 1886.30 µg/g, respectively. In addition, as shown in Fig. 3B, the contents of eriodictyol-7-O-glucoside (1), hyperoside (2), rutin (3), isoquercitrin
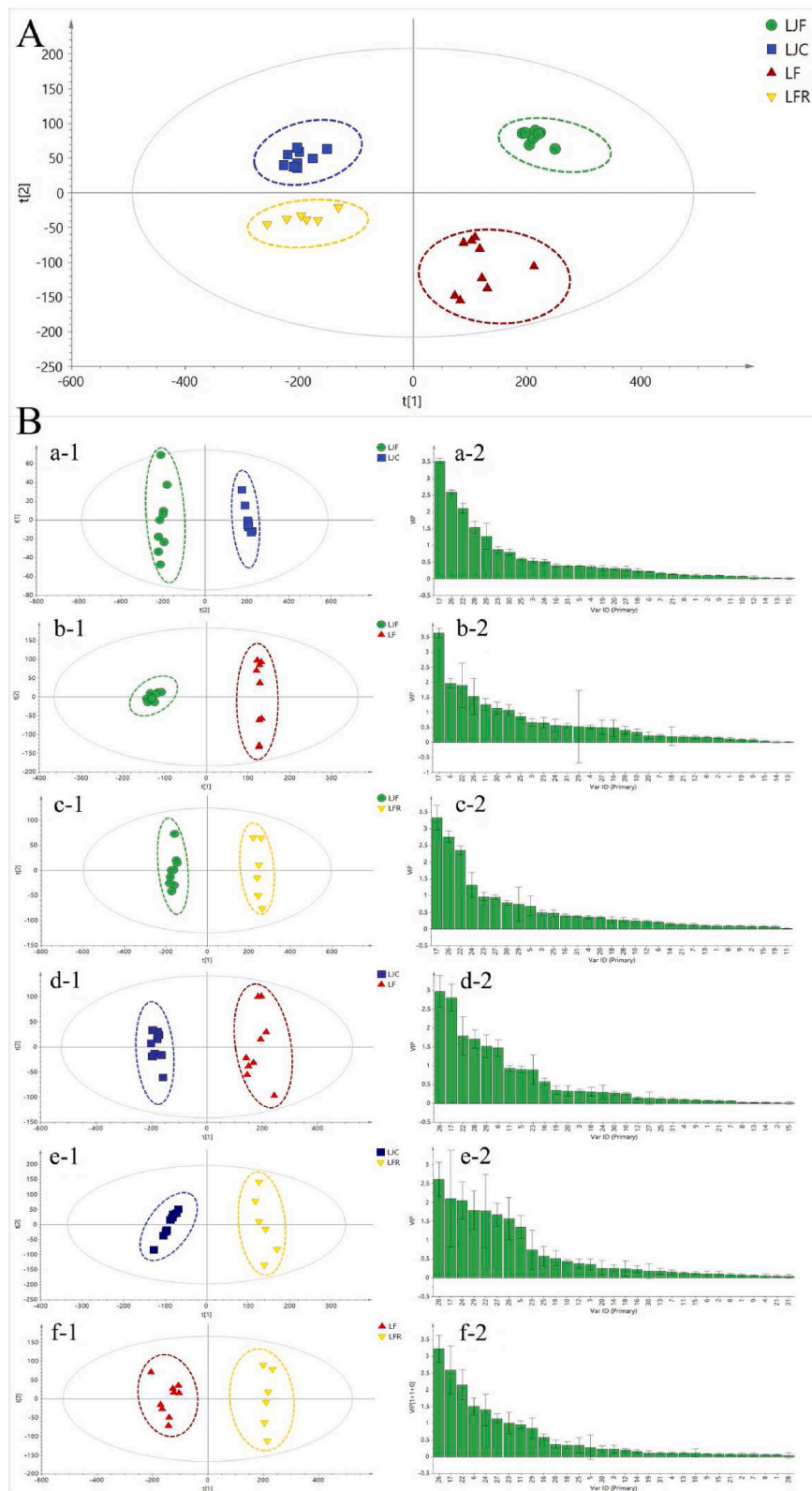
Fig. 4. Score scatter plot. (A) PCA score scatter plot of different parts of *L. japonica*. (B) The OPLS-DA (orthogonal partial least squares-discriminant analysis) score scatter plot based on a pairwise comparison (a-1, b-1, c-1, d-1, e-1, f-1), and the VIP values (a-2, b-2, c-2, d-2, e-2, f-2).

(4), kaempferol-3-O-rutinoside (7), astragalin (8), narcissin (9), chlorogenic acid (17), caffeic acid methyl ester (21), isochlorogenic acid A (22), morroniside (25), (E)-Aldosecologanin (30) and (Z)-Aldosecologanin (31) in LJF were significantly higher than those in other medicinal parts ($P < 0.05$). The contents of caffeic acid (19) and loganin (28) in LJC were significantly higher than those in other medicinal parts ($P < 0.05$). The contents of lonicerin (6), rhoifolin (11) and neochlorogenic acid (16) in LF were significantly higher than those of other medicinal parts ($P < 0.05$). The contents of luteolin (12), apigenin (13), diosmetin (14), protocatechuic acid (15), loganinic acid (24), and sweroside (27) in LFR were significantly higher ($P < 0.05$) than that of in other medicinal parts. All the 31 components to be tested were all detected in LJF, but caffeic acid methyl ester (21) was not detected in LFR, and apigenin (13) was not detected in some batches of LJC. The quantitative results were consistent with the qualitative results in Table 1. It is worth noting that protocatechuic acid (15) and caffeic acid (19) in some batches of samples were not detected quantitatively because they could not reach the quantitative limit.

In summary, the four medicinal parts of *L. japonica* contain the same components type, including phenolic acids, flavonoids, and iridoid glycosides, but they differ in composition or in the amount of some components. Chemical components are the material basis of the pharmacodynamics for TCM. According to the qualitative and quantitative results, we speculated that the differences in the composition and amount of specific components were the main reasons for the differences in the efficacy of different medicinal parts of *L. japonica*.

3.3. Multivariate statistical analysis

3.3.1. Hierarchical clustering analysis

Using the content of 31 compounds as variables and Euclidean square distance as the measurement standard, the heat map and cluster analysis were carried out by Origin software. In Fig. A.8, the depth of color reflected the value of variable, with red representing high content and blue representing low content. The results of thermogram analysis showed that the content of chemical composition in different medical part was different greatly. Among them, 18 compounds containing in LJF including chlorogenic acid, secologanic acid, isoquercitrin, etc., 11 compounds in LF including lonicerin and rhoifolin, and 8 compounds in LFR such as loganic acid, diosmetin and so on were all shown in red, which indicated that the content of these components were high. 13 compounds in LJF such as loganin and apigenin-7-O-glucoside, 29 compounds in LJC except for loganin and caffeic acid, 20 compounds in LF including protocatechuic acid and hyperoside, 23 compounds in LFR such as isochlorogenic acid A and loganic acid were all shown in blue, which indicated that the content of these components were low. The results of cluster analysis showed that all the samples were clustered 4 groups according to their medical parts, which indicated that the content of components among different medical parts differ greatly and could be used as classification basis of different medical parts.

3.3.2. Principal component analysis (PCA)

The PCA analysis was performed for distinguishing different medical parts of *L. japonica* using the content of 31 components as index. The preprocessed data was imported into SIMCA v14.1 for PCA analysis. PC1 and PC2 accounted for more than 80 %, which could be used to reflect the overall information of the sample [6] ($R^2X [1] = 0.725$, $R^2X [2] = 0.129$). The fitting degree (R^2) of the model was 97.8 %, and the prediction degree (Q^2) was 93.2 %, which indicated that the model was good and PCA could be performed.

As shown in Fig. 4A, all the samples were divided into 4 groups according to their medicinal parts and distributed in 4 quadrants, which indicated that the amount of components differed significantly among LJF, LJC, LF and LFR. The results of principal component analysis were similar to that of cluster analysis, which further verified the accuracy and reliability of the hierarchical cluster analysis.

3.3.3. Orthogonal partial least squares discriminant analysis (OPLS-DA)

For eliminating random errors unrelated to the purpose of the study, OPLS-DA analysis was performed furtherly. The results were shown in Fig. 5. The established OPLS-DA model had good fitting ($R^2X = 0.977$, $R^2Y = 0.979$) and predictability ($Q^2 = 0.971$), indicating LJF, LJC, LF and LFR were well separated. The permutation test showed that the model was not overfitted, Fig. 5B. The VIP value was used to describe the contribution of each variable to the model and explore the differential constituents for classifying different medical parts. The threshold of VIP is usually set to 1.0, which means that a compound will be selected as a potential chemical marker when the VIP value is greater than 1.0, and the higher the VIP value, the more contribution of the components to the classification of all the samples. According to the above rules, 10 compounds including chlorogenic acid (17), secologanic acid (26), isochlorogenic acid A (22), loganin (28), lonicerin (6), loganic acid (24), secoxyloganin (29), sweroside (27), luteolin (5) and rhoifolin (11) were selected as chemical markers to distinguish LJF, LJC, LF and LFR [47].

The OPLS-DA analysis was further carried out for discriminating differential components between two medical parts. As shown in Fig. 4B, the samples from the same medical pare were aggregated together and could be clearly distinguished from the samples from other medical parts. The compound with VIP >1.0 was selected as chemical marker for distinguishing different medical parts. Finally, 5 compounds including chlorogenic acid, secologanic acid, isochlorogenic acid A, loganin and secoxyloganin were selected as differential chemical markers between LJF and LJC, and the results were consistent with that reported by Cai et al. [1]. Totally 7 compounds including chlorogenic acid, lonicerin, isochlorogenic acid A, secologanic acid, rhoifolin, (E)-Aldosecologanin and luteoloside were selected for distinguishing LJF and LF. 4 compounds including chlorogenic acid, secologanic acid, isochlorogenic acid A and loganic acid were selected for screening out LJF and LFR. 6 compounds were screened out for discriminating LJC and LF, which were secologanic acid, chlorogenic acid, isochlorogenic acid A, loganin, secoxyloganin and lonicerin. 8 compounds including loganin, chlorogenic acid, loganic acid, secoxyloganin, isochlorogenic acid A, sweroside, secologanic acid and luteoloside were selected as chemical markers for distinguishing LJC and LFR, and 7 compounds including secologanic acid, chlorogenic acid, isochlorogenic acid
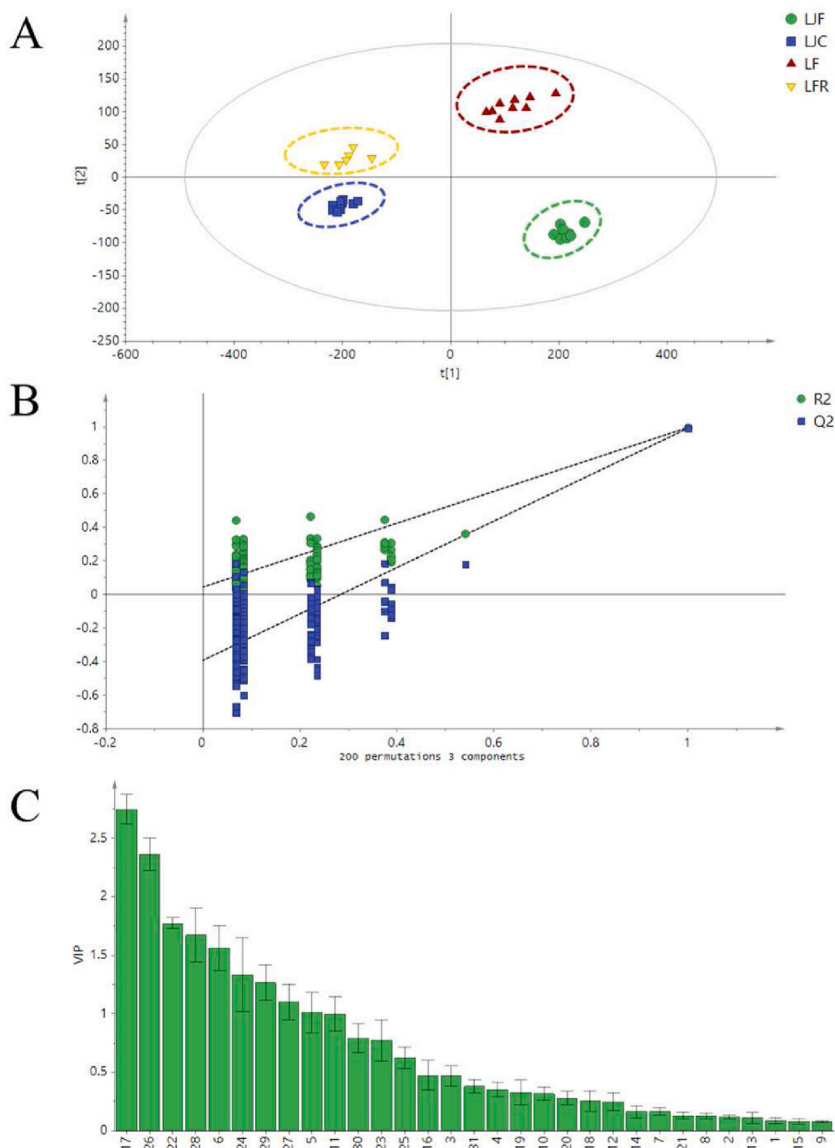
Fig. 5. The Orthogonal partial least squares discriminant analysis (OPLS-DA) score scatter plot (A), permutation test (B) and variable importance in the projection (VIP) (C) of different parts of *L. japonica*.

A, lonicerin, loganic acid, sweroside and isochlorogenic acid C were selected for distinguishing LF and LFR.

4. Discussion

LJF, LJC, LF and LFR all have the effect of heat-clearing and detoxifying, and each has its own advantages, such as LJF is better in dispersing wind heat, LJC is better in dredging wind and clearing collaterals, and LFR is better in treating intestinal wind and bloody dysentery [5]. Chemical components are the material basis of the pharmacodynamics of TCM, and the difference in efficacy is ultimately due to the difference in the components contained in it [6]. This study first screened and identified the chemical compositions of four medicinal materials by UPLC-Q-Exactive-Orbitrap-MS. And then, UPLC-QQQ-MS/MS technology was used to quantitatively analyze 31 compounds.

The qualitative results showed that a total of 86 components were characterized in the four parts, and 54 identical compounds were detected among the four parts, including 18 phenolic acids, 13 flavonoids and 16 iridoid glycosides. However, each medicinal materials had its own characteristic components, for example, 7-epi-loganin was only detected in LJC, mauritianin was only detected in LF, and cyanidin 3,5-diglucoside and peonidin 3,5-O-diglucoside were only detected in LFR. The results of quantitative analysis showed that the content of 31 components in LJF was the highest, followed by LF, LFR, and LJC. The results of multivariate statistical

analysis showed that the samples from the same medicinal parts were clustered into one group, which indicated that the samples from the same medicinal parts had the more consistent composition and content of components, while the samples from different medicinal parts differed a few greater. Therefore, we speculated that the differences in composition and content of components contained in different medicinal parts was the responsible for the differences in efficacy.

By reviewing the ancient herbal medicine, we found that in the long history of medicinal use, the medicinal parts of *L. japonica* experienced a development process from "the use of stem and leaves" to "the use of stem, leaves and flowers, but the flower is superior" [48]. In the Ming and Qing Dynasties, although stems, leaves and flowers could all be used as medicines parts, the dominance of flowers was gradually highlighted [49]. In modern times, LJF and LJC were both included in annual edition of the Chinese Pharmacopoeia, but LF have not received enough attention and only included in local standards [50]. This study showed LF contained the highest total flavonoids, and the total content of 31 components and total iridoid glycosides in LF was second only to LJF, therefore, we believe that LF have great important application value in the future.

LFR was first recorded in Yin Pian Xin Can [51]. It has the effect of clearing blood and transforming damp heat, treating intestinal wind and bloody dysentery. It is mostly used in the treatment of heat sore swelling toxin and dysentery [5]. In contrast to flowers, leaves and stems, the research on fruits is relatively lagging behind. In this study, the components of LFR were qualitatively analyzed by UPLC-Q-Exactive-Orbitrap-MS for the first time. A total of 73 components were identified, including 23 phenolic acids, 22 flavonoids and 15 iridoid glycosides. The results of quantitative analysis showed that there was no significant difference in the total content, total phenolic acid content and total iridoid glycosides content of 31 components between LJC and LFR. The content of total flavonoids in LFR was significantly higher than that of LJF and LJC, especially the contents of 6 components including luteolin, apigenin, diosmetin, protocatechuic acid, loganic acid and sweroside were significantly higher than those of other medicinal parts. Therefore, from the perspective of material basis, LFR has important development value. However, at present, the research on the pharmacological effects of LFR was not deep, only on its antibacterial [22] and antioxidant effects [23]. In the future, the research on its pharmacological activity should be increased for accelerating the development and application of LFR.

5. Conclusions

In this study, the UPLC-Q-Exactive-Orbitrap-MS technique was used for the first time for qualitatively analyzing the components contained in four medicinal materials. A total of 86 components were identified, including 25 flavonoids, 24 phenolic acids, 24 iridoid glycosides, 9 amino acids, and 4 other components. There were 54 common components among the four medicinal materials, and each medicinal material had its own unique components. The 31 components in 4 medicinal materials were quantitatively analyzed by UPLC-QQQ-MS/MS. Additionally, 10 components including chlorogenic acid, secologanic acid, isochlorogenic acid A, loganin, lonicerin, loganic acid, secoxyloganin, sweroside, luteolin and rhoifolin were the main differential components among the four parts. The research laid a foundation for the discovery of the material basis of the different efficacy of the four medicinal materials, and also provided a basis for more rational utilization of various parts of *L. japonica* and expansion of medicinal resources.

Funding statement

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xinrui Zhang: Writing – original draft, Visualization, Data curation, Conceptualization. **Xiao Yu:** Visualization, Methodology, Data curation. **Xiaomei Sun:** Visualization, Data curation. **Xianbo Meng:** Resources, Data curation. **Jian Fan:** Validation, Methodology. **Fang Zhang:** Writing – review & editing, Project administration, Funding acquisition. **Yongqing Zhang:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
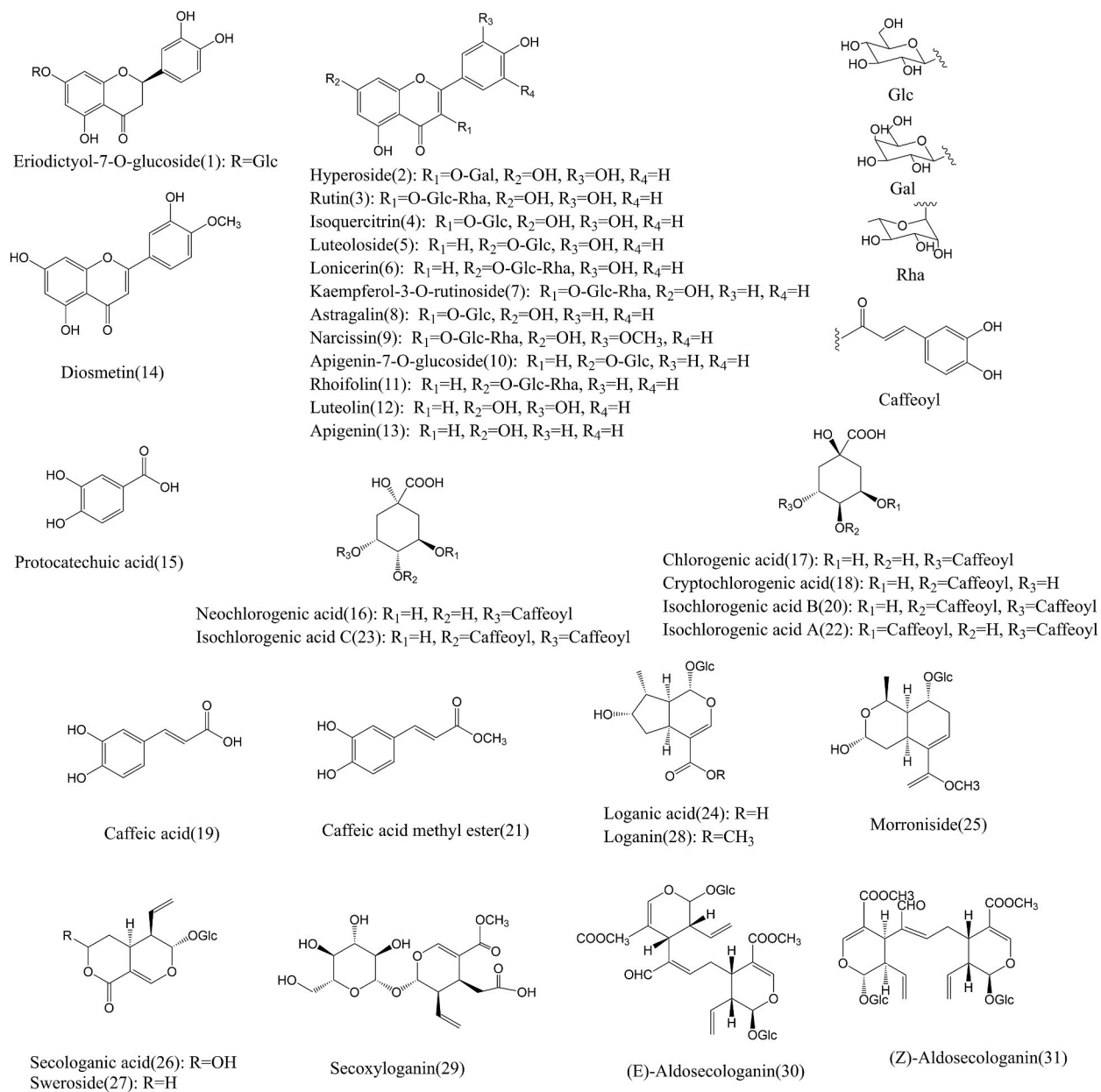
Appendixes

Eriodictyol-7-O-glucoside(1): R=Glc

Diosmetin(14)

Protocatechuic acid(15)

Hyperoside(2): R_1=O-Gal, R_2=OH, R_3=OH, R_4=H
Rutin(3): R_1=O-Glc-Rha, R_2=OH, R_3=OH, R_4=H
Isoquercitrin(4): R_1=O-Glc, R_2=OH, R_3=OH, R_4=H
Luteoloside(5): R_1=H, R_2=O-Glc, R_3=OH, R_4=H
Lonicerin(6): R_1=H, R_2=O-Glc-Rha, R_3=OH, R_4=H
Kaempferol-3-O-rutinoside(7): R_1=O-Glc-Rha, R_2=OH, R_3=H, R_4=H
Astragalin(8): R_1=O-Glc, R_2=OH, R_3=H, R_4=H
Narcissin(9): R_1=O-Glc-Rha, R_2=OH, R_3=OCH$_3$, R_4=H
Apigenin-7-O-glucoside(10): R_1=H, R_2=O-Glc, R_3=H, R_4=H
Rhoifolin(11): R_1=H, R_2=O-Glc-Rha, R_3=H, R_4=H
Luteolin(12): R_1=H, R_2=OH, R_3=OH, R_4=H
Apigenin(13): R_1=H, R_2=OH, R_3=H, R_4=H

Glc

Gal

Rha

Caffeoyl

Neochlorogenic acid(16): R_1=H, R_2=H, R_3=Caffeoyl
Isochlorogenic acid C(23): R_1=H, R_2=Caffeoyl, R_3=Caffeoyl

Chlorogenic acid(17): R_1=H, R_2=H, R_3=Caffeoyl
Cryptochlorogenic acid(18): R_1=H, R_2=Caffeoyl, R_3=H
Isochlorogenic acid B(20): R_1=H, R_2=Caffeoyl, R_3=Caffeoyl
Isochlorogenic acid A(22): R_1=Caffeoyl, R_2=H, R_3=Caffeoyl

Caffeic acid(19)

Caffeic acid methyl ester(21)

Loganic acid(24): R=H
Loganin(28): R=CH$_3$

Morroniside(25)

Secologanic acid(26): R=OH
Sweroside(27): R=H

Secoxyloganin(29)

(E)-Aldosecologanin(30)

(Z)-Aldosecologanin(31)

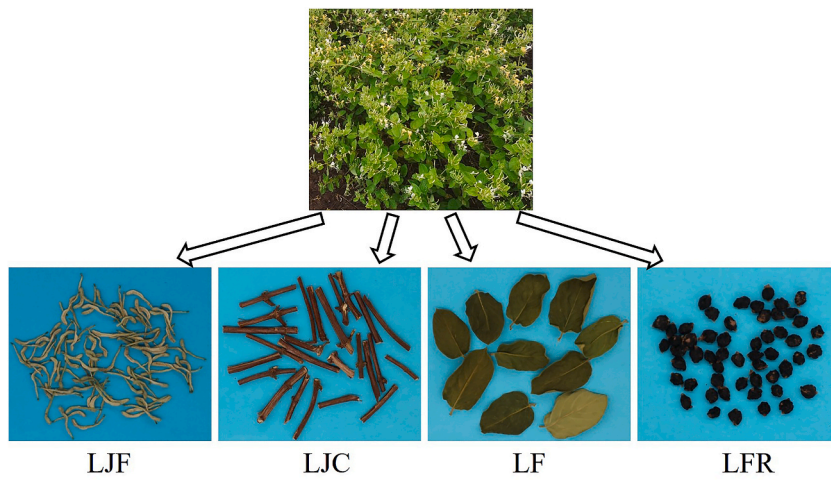**Fig. A.1.** The chemical structures of the 31 compounds.

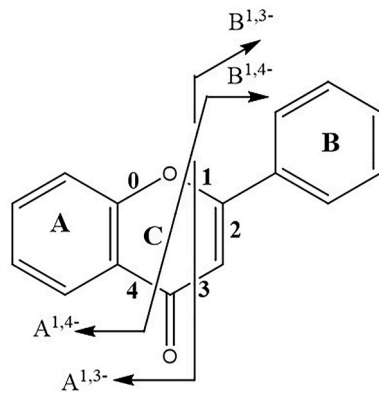**Fig. A.2.** Four segments (LJF, LJC, LF, and LFR) of *L. japonica.*

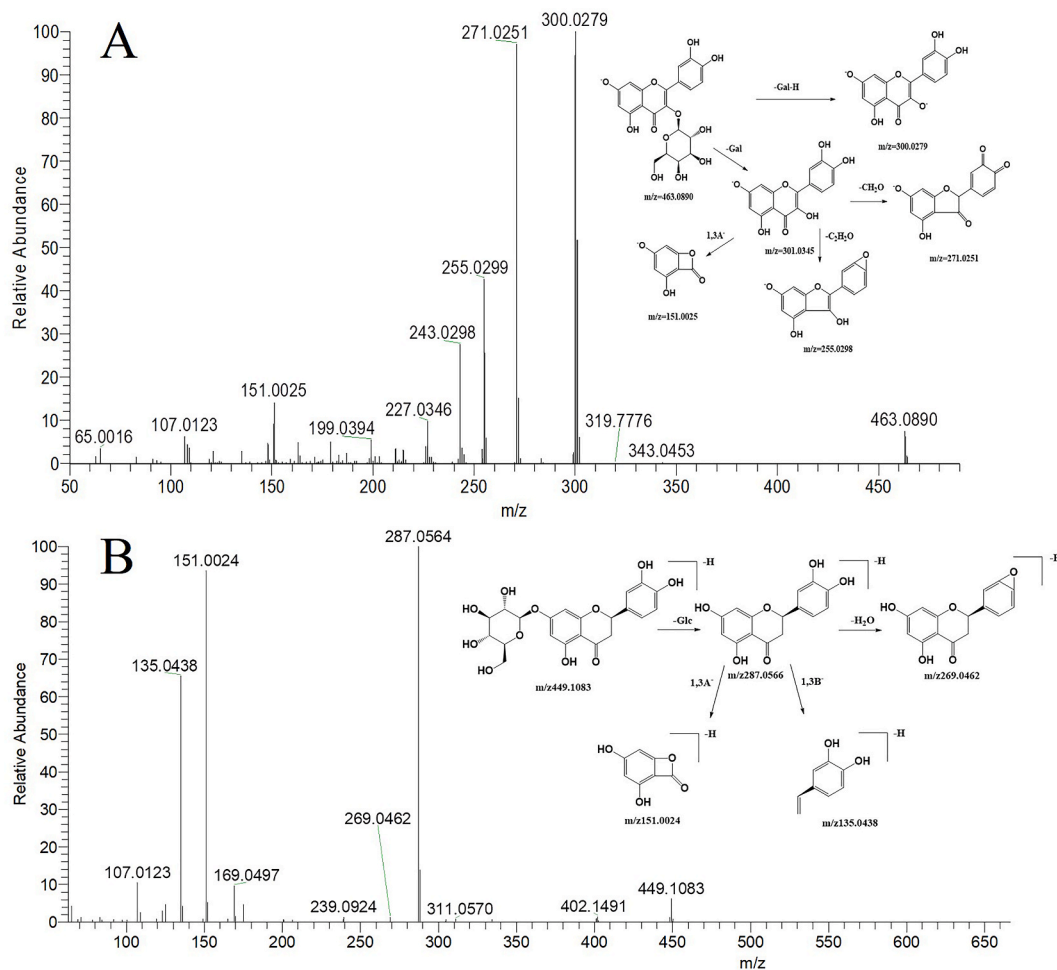**Fig. A.3.** Schematic diagram of the fracture site of flavonoid aglycone in negative ion mode.

Fig. A.4. The possible fragmentation pathways of hyperoside (A) and eriodictyol-7-O-glucoside (B) in *L. japonica*.
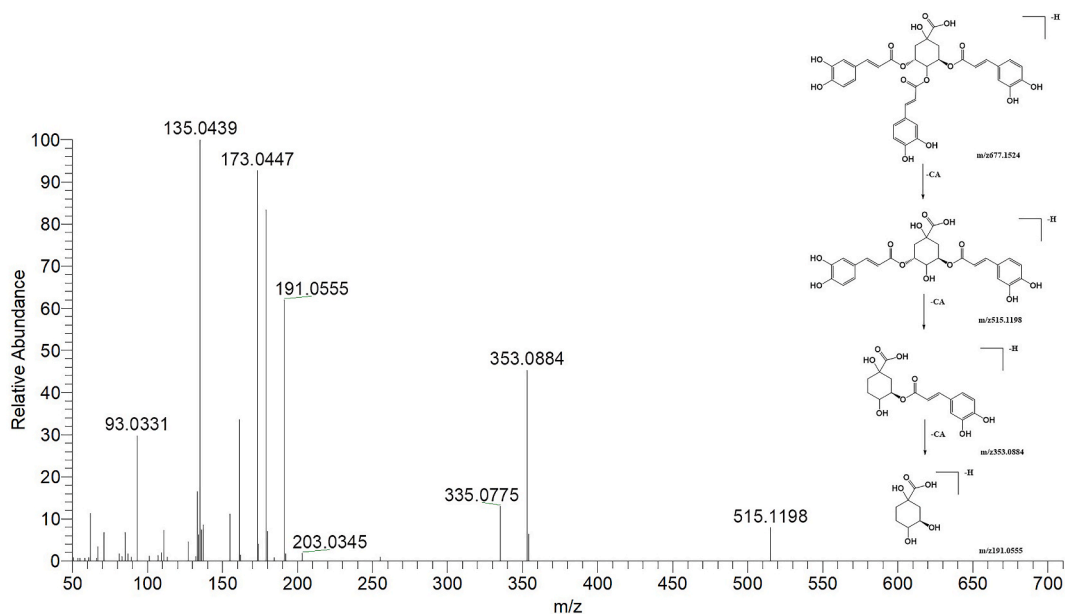

Fig. A.5. The possible fragmentation pathways of 3,4,5-tricaffeoylquinic acid in *L. japonica*.
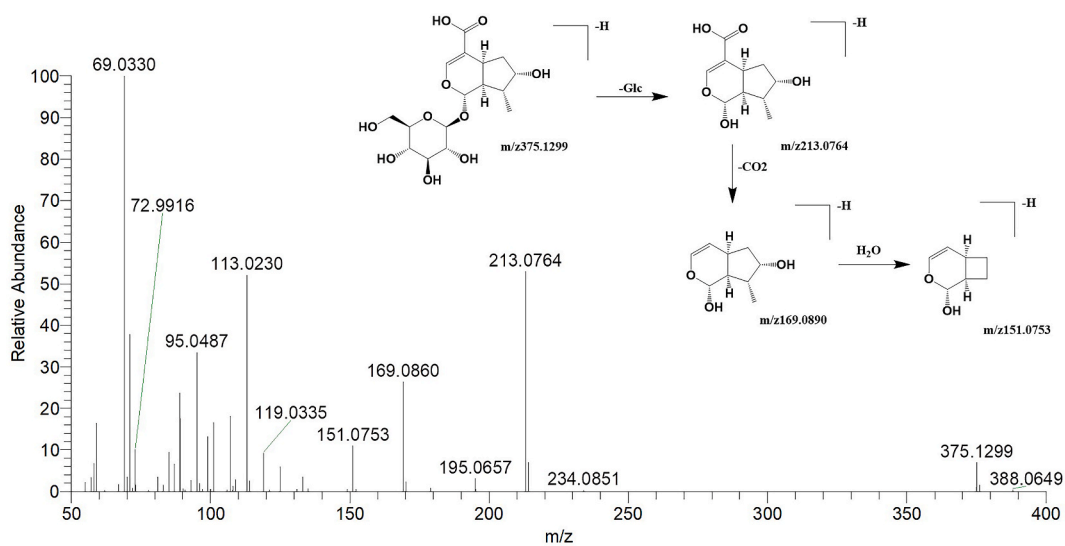
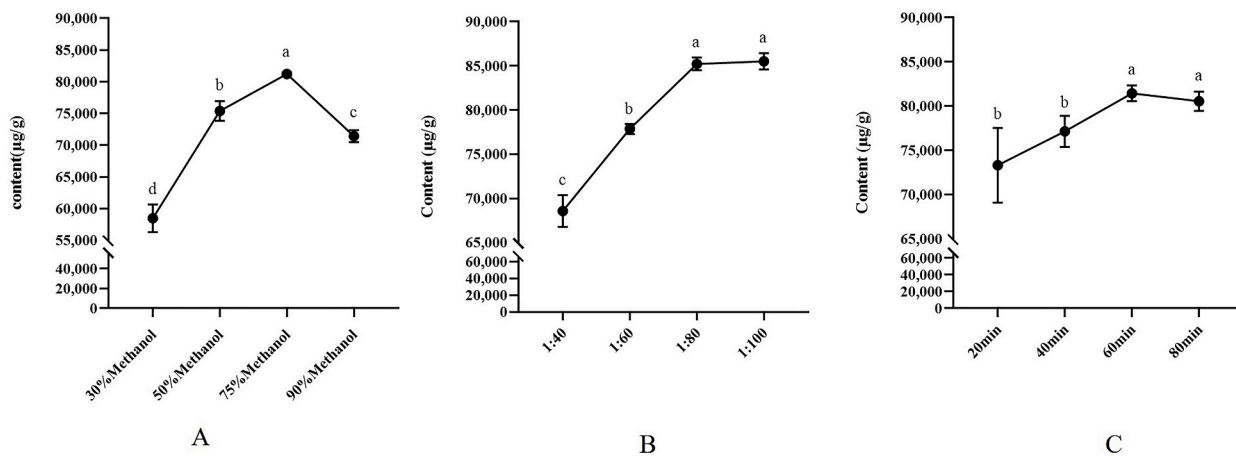**Fig. A.6.** The possible fragmentation pathways of loganic acid in *L. japonica*.

Fig. A.7. Optimization of different extraction conditions: (A) extraction solvent, (B) solid-liquid ratio, (C) extraction time (Different lowercase letters indicate a significant difference, $P < 0.05$).
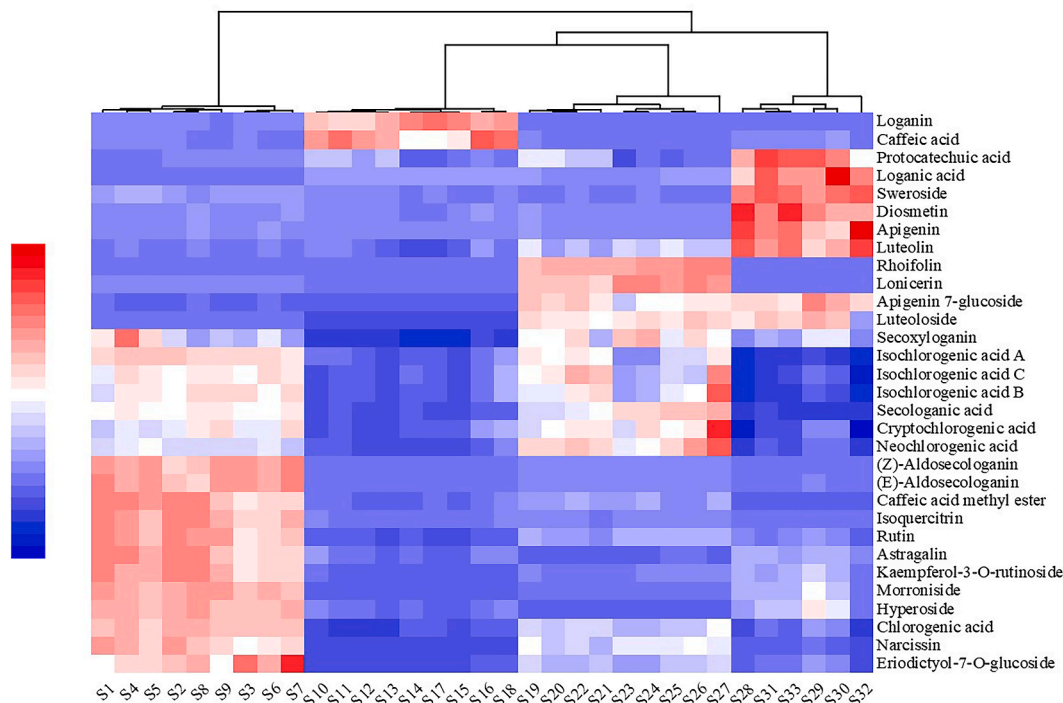
Fig. A.8. Hierarchical clustering heat map.

Table A1
Regression equations, limits of detections (LODs), limits of quantifications (LOQs), intra- and inter-day precisions, repeatability, stabilities, and recoveries of 31 compounds

NO.	Compounds	Regrseeion Equation	r	Linear Range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Precision		Repeatability (RSD%; n = 6)	Stability (RSD%; n = 6)	Recovery	
							Intra-Day (RSD %; n = 6)	Inter-Day (RSD %; n = 3)			Mean	RSD %
1	Eriodictyol-7-O-glucoside	y = 841,458x + 4461.7	0.9993	6.53-1045.2	0.18	0.59	2.45	1.76	4.69	4.96	98.95	2.48
2	Hyperoside	y = 805,916x − 5827.6	0.9999	19.08-7630	0.51	1.69	4.51	1.91	4.02	4.61	101.44	5
3	Rutin	y = 629,355x − 32,795	0.9996	52-13,000	0.19	0.64	2.47	2.16	2.57	3.05	97.75	3.25
4	Isoquercitrin	y = 750,010x + 50,731	0.9997	77.63-12,420	0.61	2.04	2.07	2.17	3.56	2.96	96.78	3.78
5	Luteoloside	y = 10^6x + 949,497	0.9973	91.6-36,640	0.34	1.14	1.22	1.99	2.12	2.34	98.29	2.37
6	Lonicerin	y = 488,451x + 612,980	0.998	98.1-98,100	0.62	2.06	1.1	0.41	2.74	3.27	96.95	2.68
7	Kaempferol-3-O-rutinoside	y = 768,831x − 3033	0.9992	13.88-2220	0.12	0.4	3.77	2.68	2.68	2.03	97.58	3.17
8	Astragalin	y = 477,373x − 1263.8	0.9993	13.26-1326	0.17	0.55	2.91	2.05	1.94	1.32	99.4	3.53
9	Narcissin	y = 967,613x − 478.47	0.9997	2.5-624	0.13	0.43	4.44	3.33	2.98	2.17	98.89	3.1

(continued on next page)

Table A1 (*continued*)

NO.	Compounds	Regrseeion Equation	r	Linear Range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Precision		Repeatability (RSD%; n = 6)	Stability (RSD%; n = 6)	Recovery	
							Intra-Day (RSD%; n = 6)	Inter-Day (RSD%; n = 3)			Mean	RSD%
10	Apigenin 7-glucoside	y = 2×10ˆ6x + 39,023	0.9996	5.03-4020	0.08	0.26	1.55	2.23	2.89	2.59	99.72	4.06
11	Rhoifolin	y = 576,956x + 143,585	0.9993	25.71-41,140	0.08	0.26	0.96	1.09	2.26	1.99	95.11	2.43
12	Luteolin	y = 10ˆ6x + 123,272	0.999	88.4-4420	0.36	1.2	1.88	3.21	4.54	3.55	100.25	4.27
13	Apigenin	y = 886,383x + 7330	0.9993	5.88-1176	0.13	0.45	2.16	3.27	5.17	3.45	100.86	4.4
14	Diosmetin	y = 10ˆ6x + 15,221	0.999	15.83-1582.5	0.01	0.03	1.46	1.73	4.93	1.72	99.3	4.76
15	Protocatechuic acid	y = 371,665x + 4840.6	0.9993	25.63-410	2.67	8.91	1.92	1.29	4.22	3.55	95.86	4.42
16	Neochlorogenic acid	y = 313,347x − 24,517	0.9998	594-23,760	8.91	29.7	2.75	0.88	1.93	2.63	96.91	3.24
17	Chlorogenic acid	y = 222,890x + 7×10ˆ6	0.9981	12480-624,000	5.73	19.11	1.96	2.28	2.3	3.15	98.72	1.05
18	Cryptochlorogenic acid	y = 240,199x − 8602.8	0.9998	370-18,500	2.88	9.59	1.63	1.74	3.08	2.97	100.24	1.93
19	Caffeic acid	y = 208,723x + 12,244	0.999	60.5-6050	3.49	11.63	2.22	1.5	3.91	3.28	97.58	3.95
20	Isochlorogenic acid B	y = 344,937x + 6967.3	0.9992	44.2-11,050	4.42	14.73	1.38	1.92	2.8	2.52	96.78	1.55
21	Caffeic acid methyl ester	y = 320,820x + 12,632	0.999	2.44-1950	0.33	1.11	2.29	1.16	5.47	5.13	95.9	3.78
22	Isochlorogenic acid A	y = 385,953x +10ˆ6	0.9966	706.2-282,480	2.14	7.12	2.57	1.8	1.73	1.48	99.23	2.5
23	Isochlorogenic acid C	y = 531,005x + 10ˆ6	0.9987	681-68,100	4.5	15	1.46	0.57	2.38	3.67	98.52	2.23
24	Loganic acid	y = 168,944x + 29,919	1	699-111,840	0.47	1.57	2.3	3.19	2.39	2.34	99.04	3.76
25	Morroniside	y = 221,679x + 14,103	0.9998	149.5-23,920	0.56	1.87	2.97	2.83	4.69	3.76	100.48	3.71
26	Secologanic acid	y = 58,264x + 629,888	0.9959	1110-444,000	2.17	7.22	1.59	3.57	2.59	2.64	96.53	3.84
27	Sweroside	y = 54,180x + 60,710	0.9998	1896-75,840	11.85	39.5	1.89	1.8	2.45	1.96	95.25	2.14
28	Loganin	y = 70,194x + 24,175	1	73.95-118,320	1.54	5.14	3.16	3.43	2.75	3.55	95.68	3.97
29	Secoxyloganin	y = 36,943x − 7256.1	0.9997	1352-135,200	5.87	19.57	1.08	0.37	1.77	3.29	98.81	1.12
30	(E)-Aldosecologanin	y = 61,645x + 35,945	0.9997	400-40,000	1.89	6.3	1.5	1.74	3.13	2.85	97.44	2.02
31	(Z)-Aldosecologanin	y = 59,573x − 851.9	1	83.25-8325	0.93	3.08	2.2	1.83	3.72	3.04	96.4	2.47

Table A2

Contents of 31 compounds in samples of LJF, LJC, LF and LFR (μg/g, mean ± SD , n = 3).

References

[1] Z. Cai, C. Wang, L. Zou, X. Liu, J. Chen, M. Tan, Y. Mei, L. Wei, Comparison of multiple bioactive constituents in the flower and the caulis of Lonicera japonica based on UFLC-QTRAP-MS/MS combined with multivariate statistical analysis, Molecules 24 (2019) 1936, https://doi.org/10.3390/molecules24101936.

[2] Chinese Pharmacopoeia Commission, Pharmacopoeia of the people's Republic of China, Beijing 1, Chinese Medical Science and Technology Press, 2020, p. 273.

[3] Chinese Pharmacopoeia Commission, Pharmacopoeia of the people's Republic of China, Beijing 1, Chinese Medical Science and Technology Press, 2020, p. 207.

[4] H. Zhu, J. Chen, L. Wen, Z. Wang, F. Jing, D. Wang, Research advances on chemical constituents of leaves from *Lonicera japonica* Thunb, Shandong Sci. 29 (2016) 30–39, https://doi.org/10.3969/j.issn.1671-5217.2017.01.143.

[5] J. Li, Y. Han, Research advances on non - flower bud parts of *Lonicera japonica* thunb, Chinese Archives of Traditional Chinese Medicine 38 (2020) 43–50, https://doi.org/10.13193/j.issn.1673-7717.2020.04.010.

[6] S. Yin, Y. Mei, L. Wei, L. Zou, Z. Cai, N. Wu, J. Yuan, X. Liu, H. Ge, D. Wang, D. Wang, Comparison of multiple bioactive constituents in the corolla and other parts of *abelmoschus manihot*, Molecules 26 (2021) 1864, https://doi.org/10.3390/molecules26071864.

[7] J. Liu, S. Chen, A. Liu, Material basis analysis of "homologous and different effect" of Lotus leaf and Lotus plumule, Chin. J. Exp. Tradit. Med. Formulae 26 (2020) 131–139, https://doi.org/10.13422/j.cnki.syfjx.20201548.

[8] Z. Zhang, J. Liu, Q. Fan, Y. Liu, Y. Wu, J. Zhou, F. He, Comparative analysis and research on composition of mulberry-sourced medicinal materials by fingerprint division total statistical moment method and information entropy, China J. Chin. Mater. Med. 46 (2021) 2547–2555, https://doi.org/10.19540/j.cnki.cjcmm.20200924.203.

[9] Y. Zhang, X. Huang, F. Zhao, Y. Tang, L. Yin, Study on the chemical markers of Caulis Lonicerae japonicae for quality control by HPLC-QTOF/MS/MS and chromatographic fingerprints combined with chemometrics methods, Anal. Methods 7 (2015) 2064–2076, https://doi.org/10.1039/C4AY02744B.

[10] S. Liu, L. Yang, S. Wang, J. Pan, Simultaneous determination of 9 main components of *Lonicera japonica* thunb. By UPLC-MS/MS and analysed combine with chemometrics, Nat. Prod. Commun. 15 (2020) 1–8, https://doi.org/10.1177/1934578X20953272.

[11] H. Pan, H. Zhou, S. Miao, J. Cao, J. Liu, L. Lan, Q. Hu, X. Mao, S. Ji, An integrated approach for global profiling of multi-type constituents: comprehensive chemical characterization of Lonicerae Japonicae Flos as a case study, J. Chromatogr. A 1613 (2020) 460674, https://doi.org/10.1016/j.chroma.2019.460674.

[12] L. Yan, Y. Xie, Y. Wang, J. Zhu, M. Li, X. Liu, D. Zhao, Variation in contents of active components and antibacterial activity in different parts of *Lonicera japonica* Thunb, Asian Biomed. 14 (2020) 19–26, https://doi.org/10.1515/abm-2020-0004.

[13] X. Guo, Y. Wang, X. Yu, R. Yang, L. Wang, F. Zhang, Y. Zhang, Simultaneous determination of 11 active components in *Lonicera japonica* flowers and leaves at different development stages by HPLC-DAD, China J. Chin. Mater. Med. 47 (2022) 2148–2157, https://doi.org/10.19540/j.cnki.cjcmm.20220114.101.

[14] J. Ye, J. Su, K. Chen, H. Liu, X. Yang, Y. He, W. Zhang, Comparative investigation on chemical constituents of flower bud, stem and leaf of *Lonicera japonica* Thunb. by HPLC-DAD-ESI-MS/MS (n) and GC-MS, J. Anal. Chem. 69 (2014) 777–784, https://doi.org/10.1134/s1061934814080036.

[15] Y. Wang, Y. He, Z. Dai, S. Kang, J. Zhang, S. Ma, A comparative study on bioactive constituents in different parts of *Lonicera japonica* determined by HPLC-ESI-MS(n), J. Asian Nat. Prod. Res. 18 (2016) 988–1003, https://doi.org/10.1080/10286020.2016.1205039.

[16] R. Li, X. Kuang, W. Wang, C. Wan, W. Li, Comparison of chemical constitution and bioactivity among different parts of *Lonicera japonica* Thunb, J. Sci. Food Agric. 100 (2020) 614–622, https://doi.org/10.1002/jsfa.10056.

[17] J. Xiong, S. Li, W. Wang, Y. Hong, K. Tang, Q. Luo, Screening and identification of the antibacterial bioactive compounds from Lonicera japonica Thunb. Leaves, Food Chem. 138 (2013) 327–333, https://doi.org/10.1016/j.foodchem.2012.10.127.

[18] D. Wang, Study on Chemical Constituents of Honeysuckle Leaves and Their Resistance to H-5 Subtype Avian Influenza Virus, Ph.D. Thesis, Shandong Agricultural University, 2013.

[19] J. Zhu, D. Zhou, D. Wu, W. Liu, X. Liu, TiO$_2$ nanotube immobilised 5-lipoxygenase-mediated screening and isolation of anti-inflammatory active compounds from the leaves of *lonicera japonica* thunb, J. Enzym. Inhib. Med. Chem. 37 (2022) 2540–2550, https://doi.org/10.1080/14756366.2022.2121392.

[20] K. Yan, S. Zhao, L. Bian, X. Chen, Saline stress enhanced accumulation of leaf phenolics in honeysuckle (*Lonicera japonica* Thunb.) without induction of oxidative stress, Plant Physiol. Biochem. 112 (2017) 326–334, https://doi.org/10.1016/j.plaphy.2017.01.020.

[21] J. Yu, K. Wang, H. Zhao, L. Chen, X. Wang, Bioactive constituents from the leaves of *Lonicera japonica*, Fitoterapia 162 (2022) 105277, https://doi.org/10.1016/j.fitote.2022.105277.

[22] K. Yang, Y. Han, Q. Sun, F. Zhang, Z. Peng, Q. Liu, J. Li, Relationship between spectrum -antibacterial effect of fruits of rendong (*Lonicera japonica* thunb.), Chinese Archives of Traditional Chinese Medicine 40 (2022) 114, https://doi.org/10.13193/j.issn.1673-7717.2022.07.029, 117+268-270.

[23] H. He, Q. Guo, X. Lu, Study on antioxidant activity of fruits of *Lonicera japonica* thunb, Shandong Agricultural Sciences 51 (2019) 61–64, https://doi.org/10.14083/j.issn.1001-4942.2019.04.011.

[24] S. Liu, M. Su, Study of Lonicerae japonicae flos from genuine producing areas of Shandong on rapid resolution liquid chromatography fingerprint, China Journal of Traditional Chinese Medicine and Pharmacy 30 (2015) 2557–2560.

[25] T. Liu, L. Yang, C. Dong, Q. Gao, D. Qi, X. Xu, J. Li, Study on the fingerprint and composition changes of Lonicerae japonicae flos at different harvest periods, J. Chin. Med. Mater. 44 (2021) 2358–2362, https://doi.org/10.13863/j.issn1001-4454.2021.10.020.

[26] P. Li, C. Li, M. Liu, D. Wang, Y. Bai, L. Huang, Z. Wu, W. Peng, W. Sui, Systematic analysis of chemical constitue of Lonicerae japonicae flos and Lonicerae flos by UFLC-triple-Q-TOF-MS/MS, Central South Pharmacy 14 (2016) 363–369.

[27] H. Duan, Study on the Production Quality Standard of Lonicerae Japonicae Flos, the Raw Material of Reduning Injection, Nanjing University of Chinese Medicine, 2020. M.S. thesis.

[28] Y. Zhang, X. Huang, F. Zhao, Y. Tang, L. Yin, Study on the chemical markers of Caulis Lonicerae japonicae for quality control by HPLC-QTOF/MS/MS and chromatographic fingerprints combined with chemometrics methods, Anal. Methods 7 (2015) 2064–2076, https://doi.org/10.1039/c4ay02744b.

[29] Z. Cai, H. Liao, C. Wang, J. Chen, M. Tan, Y. Mei, L. Wei, H. Chen, R. Yang, X. Liu, A comprehensive study of the aerial parts of *Lonicera japonica* Thunb. based on metabolite profiling coupled with PLS-DA, Phytochem. Anal. 31 (2020) 786–800, https://doi.org/10.1002/pca.2943.

[30] A.Z. Kucharska, A. Sokół-Łętowska, J. Oszmiański, N. Piórecki, I. Fecka, Iridoids, phenolic compounds and antioxidant activity of edible honeysuckle berries (Lonicera caerulea var. kamtschatica sevast.), Molecules 22 (2017) 405, https://doi.org/10.3390/molecules22030405.

[31] A.Z. Kucharska, I. Fecka, Identification of iridoids in edible honeysuckle berries (Lonicera caerulea L. Var. kamtschatica sevast.) by UPLC-ESI-qTOF-MS/MS, Molecules 21 (2016) 1157, https://doi.org/10.3390/molecules21091157.

[32] L. Xiong, L. Zhang, J. Li, G. Pu, L. Zhang, H. Yang, Y. Zhang, Extraction optimization and composition distribution of total phenolic acids from Lonicerae japonicae flos, Chin. Med. Mat. 8 (2022) 1929–1935, https://doi.org/10.13863/j.issn1001-4454.2022.08.029.

[33] R. Fujita, T. Hayasaka, S. Jin, S.P. Hui, Y. Hoshino, Comparison of anthocyanin distribution in berries of Haskap (*Lonicera caerulea* subsp. *edulis* (Turcz. ex. Herder) Hultén), Miyama-uguisukagura (*Lonicera gracilipes* Miq.), and their interspecific hybrid using imaging mass spectrometry, Plant Sci. 300 (2020) 110633, https://doi.org/10.1016/j.plantsci.2020.110633.

[34] M. Ren, J. Chen, Y. Song, L. Sheng, P. Li, L. Qi, Identification and quantification of 32 bioactive compounds in Lonicera species by high performance liquid chromatography coupled with time-of-flight mass spectrometry, J. Pharm. Biomed. Anal. 48 (2008) 1351–1360, https://doi.org/10.1016/j.jpba.2008.09.037.

[35] X. Zhang, Q. Guo, B. Yu, M. Li, L. Huo, J. Wang, Qualitative analysis of the chemical constituents of *Lonicera japonica* and its tetraploid cultivar Jiufengyihao, J. Chin. Pharmaceut. Sci. 24 (2015) 809–815.

[36] M.A. Álvarez-Fernández, A.B. Cerezo, A.M. Cañete-Rodríguez, A.M. Troncoso, M.C. García-Parrilla, Composition of nonanthocyanin polyphenols in alcoholic-fermented strawberry products using LC-MS (QTRAP), high-resolution MS (UHPLC-Orbitrap-MS), LC-DAD, and antioxidant activity, J. Agric. Food Chem. 63 (2015) 2041–2051, https://doi.org/10.1021/jf506076n.

[37] W. Li, H. Wang, L. Zeng, G. Zhang, Y. Lin, Characterization of flavonoids from five species of Ardisia using UHPLC-Q-orbitrap-MS/MS, tradit, Chin. Drug Res. Clin. Pharmacol. 33 (2022) 91–96, https://doi.org/10.19378/j.issn.1003-9783.2022.01.013.

[38] C.L.G. Santos, C.F.F. Angolini, K.O.G. Neves, E.V. Costa, A.D.L. de Souza, M.L.B. Pinheiro, H.H.F. Koolen, F.M.A. da Silva, Molecular networking-based dereplication of strictosidine-derived monoterpene indole alkaloids from the curare ingredient *Strychnos peckii*, Rapid Commun. Mass Spectrom. 34 (2020) e8683, https://doi.org/10.1002/rcm.8683.

[39] X. Li, X. Sun, S. Cai, X. Ying, F. Li, Investigation on the chemical constituents and variation of the flower buds of Lonicera species by UPLC-ESI-MS/MS and principle component analysis, Acta Pharm. Sin. 44 (2009) 895–904, https://doi.org/10.16438/j.0513-4870.2009.08.008.

[40] Y. Wang, L. Li, W. Ji, S. Liu, J. Fan, H. Lu, X. Wang, Metabolomics analysis of different tissues of *Lonicera japonica* thunb. Based on liquid chromatography with mass spectrometry, Metabolites 13 (2023) 186, https://doi.org/10.3390/metabo13020186.

[41] C. Fu, P. Yu, M. Wang, F. Qiu, Phytochemical analysis and geographic assessment of flavonoids, coumarins and sesquiterpenes in *Artemisia annua* L. based on HPLC-DAD quantification and LC-ESI-QTOF-MS/MS confirmation, Food Chem. 312 (2020) 126070, https://doi.org/10.1016/j.foodchem.2019.126070.

[42] Y. Li, H. Dai, M. Wang, X. Li, L. Wang, M. Xue, Elucidation of quercetin by electron spray ionization mass spectrometry, J. Chin. Mass Spectrom. Soc. 30 (2009) 374–378.

[43] X. Gong, W. Liu, L. Cao, J. Yu, D. Si, J. Li, P. Tu, J. Li, Y. Song, Rapid chemome profiling of chemical components of Lonicerae Japonicae Flos using DI-MS/MSALL, China J. Chin. Mater. Med. 46 (2021) 2220–2228, https://doi.org/10.19540/j.cnki.cjcmm.20210220.302.

[44] L. Li, Y. Li, H. Lu, X. Wang, Metabolomics analysis of *Lonicera japonica* in different flowering stages based on liquid chromatography-mass spectrometry, J. Instrum. Anal. 39 (2020) 1501–1507.

[45] Y. Mei, L. Wei, M. Tan, C. Wang, L. Zou, J. Chen, Z. Cai, S. Yin, F. Zhang, C. Shan, X. Liu, Qualitative and quantitative analysis of the major constituents in Spatholobi Caulis by UFLC-Triple TOF-MS/MS and UFLC-QTRAP-MS/MS, J. Pharm. Biomed. Anal. 194 (2021) 113803, https://doi.org/10.1016/j.jpba.2020.113803.

[46] D.M. Zhang, Phenolic Acid Chemistry, first ed., Chemical Industry Press, Beijing, 2009.

[47] Z. Du, J. Li, X. Zhang, J. Pei, L. Huang, An integrated LC-MS-based strategy for the quality assessment and discrimination of three *panax* species, Molecules 23 (2018) 2988, https://doi.org/10.3390/molecules23112988.

[48] C. Xue, Intension research on influencing factors of medicinal properties formation of *Lonicera japonica* and cause hypothesis in explaining medicinal properties of Chinese materia medica single herb, Chin. Tradit. Herb. Drugs 51 (2020) 3090–3096, https://doi.org/10.7501/j.issn.0253-2670.2020.11.029.

[49] T. Liu, C. Dong, Q. Gao, J. Zhang, D. Qi, X. Xu, J. Li, Brief discussion on textual research ideas of ancient books and textual collection of predecessors: a case study of *Lonicera japonica*, Chin. Tradit. Herb. Drugs 52 (2021) 2163–2177, https://doi.org/10.7501/j.issn.0253-2670.2021.07.033.

[50] Shandong Food and Drug Administration, Shandong Province Chinese Herbal Medicine Standard, Shandong Science and Technology Press Co., Ltd, 2012, 2013.

[51] Y. Wang, Yin Pian Xin Can, Shanghai Qianqingtang Book Company, Shanghai, 1936, p. 128.