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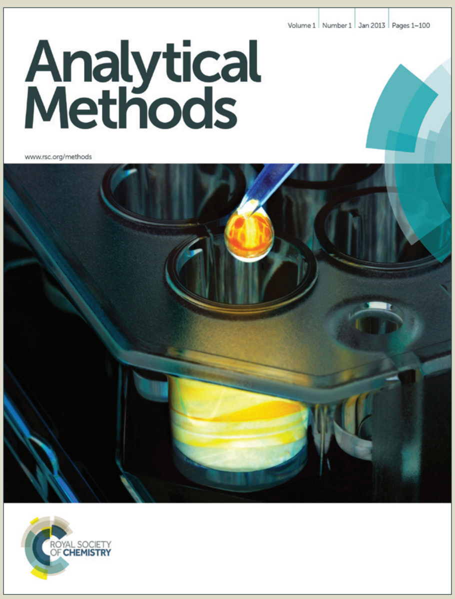

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HPLC-LTQ-Orbitrap MS^n profiling method to comprehensively characterize multiple chemical constituents in Xiao-er-qing-jie granules

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Abbreviations: HPLC-LTQ-Orbitrap, high performance liquid chromatography coupled with a linear ion trap-orbitrap mass spectrometry; XEQJ, Xiao-er-qing-jie granules; TCM, traditional Chinese medicine; ESI, electrospray ionization; CID, collision induced dissociation; FLJ, Flos Lonicerae Japonicae; FF, Forsythiae Fructus; CL, Cortex Lycii; IN, Indigo Naturalis; CARR, Cynanchi Atrati Radix et Rhizoma; RRP, Radix Rehmanniae Praeparata; PH, Pogostemonis Herba; HRMS, high-resolution mass spectrometry; CE, collision energy; TIC, total ion chromatograms; EIC, extracted ion chromatogram.

ABSTRACT

In the present study, a high performance liquid chromatography coupled with a linear ion trap-orbitrap mass spectrometry (HPLC-LTQ-Orbitrap) method was developed for a comprehensive study of the multiple chemical constituents in Xiao-er-qing-jie granules (XEQJ), which is regularly used as traditional Chinese medicine (TCM) for the treatment of children with high fever, sore throat, and lusterless complexion. Seven major categories of constituents preliminarily isolated from the component herbs were rapidly characterized using HPLC-LTQ-Orbitrap. The fragmentation patterns of these compounds with different skeleton were clearly elaborated in the electrospray ionization (ESI) collision induced dissociation (CID)-MS/MS experiments. Based on the accurate mass measurement (< 5 ppm), MS/MS fragmentation patterns, diagnostic product ions, and different chromatographic behavior, 91 compounds were unambiguously identified or tentatively characterized, including 33 phenylethanoid glycosides, 13 phenolic acids, 11 flavonoids, 10 alkaloids, 9 ligans, 9 iridoid glycosides, and 6 saponins. Among them, 2 compounds were potential new ones from Forsythiae Fructus and 24 were unambiguously confirmed by comparing with their respective reference standards. The results demonstrated that our established method was useful and efficient to screen and identify targeted constituents from TCM extracts and other organic matter mixtures whose compounds contained can also be classified into families on the basis of the common carbon skeletons.

Keywords: Xiao-er-qing-jie granules; HPLC-LTQ-Orbitrap mass spectrometer; Chemical constituents; Identification; Fragmentation pathway

1. Introduction

Xiao-er-qing-jie granules (XEQJ), which is officially listed in the Drug Standard of Ministry of Health of the People's Republic of China, is a regularly used traditional Chinese medicine (TCM) for the treatment of children with high fever, sore throat, and lusterless complexion.[1–2] The recipe of XEQJ is composed of eight herbal medicines, *viz.*, Flos Lonicerae Japonicae (FLJ) (750g), Forsythiae Fructus (FF) (750 g), Cortex Lycii (CL) (750 g), Indigo Naturalis (IN) (250 g), Cynanchi Atrati Radix et Rhizoma (CARR) (750 g), Radix Rehmanniae Praeparata (RRP) (750 g), Pogostemonis Herba (PH) (750 g), and Gypsum Fibrosum (1250 g).[3]

Although the chemical constituents in the component herbs of XEQJ have been intensively studied,[4–7] little is known about the chemical composition of XEQJ, and few reports are available on its quality control. For example, Huang *et al.* determined the contents of chlorogenic acid and phillyrin in XEQJ.[8] These two chemical compositions were from only two herbs, and could not comprehensively control the quality of XEQJ. Therefore, to develop a profiling and reliable method to screen and characterize the multiple chemical constituents in XEQJ would be an important step towards further understanding of its pharmacological effects and ultimately quality control.

With the rapid development of high performance liquid chromatography / electrospray ionization tandem mass spectrometry (HPLC/ESI-MS^n), it has played an increasingly important role in screening and identification of natural products in plant extracts in recent years.[9–12] Especially HPLC coupled with high-resolution MS (HPLC/HRMS), which can give exact mass and has become an extremely powerful tool for characterization phytochemical compounds of different structure types from complex matrices with its high resolution and best sensitivity, including flavonoids, saponins, phenolic acids, and alkaloids.[13–16] For example, the hybrid linear ion trap orbitrap mass spectrometer (LTQ-Orbitrap) combined high trapping capacity and MS^n scanning function of the linear ion trap along with accurate mass measurements within 5 ppm and a resolving power of up to 100000 over a wider dynamic range compared to many other mass spectrometers.[17] In particular, orbitrap facilitated a fast data-dependent acquisition of accurate MS^n spectra on a LC timescale, these advantages could be used for increasing the throughput and identification efficiency of compounds in TCMs. Here, we first systematically reported the structural characterization on the various chemical constituents of XEQJ by using HPLC-LTQ-Orbitrap MS. First, the collision induced dissociation (CID)-MS/MS fragmentation pathways of some certain constituents ever isolated from XEQJ were proposed and then the diagnostic product ions corresponding to a certain substructure or substituent group were deduced, which could be applied to the structural characterization of serial compounds that have not reported yet. By comparing the fragmentation patterns, retention time, MS^n data with those of the reference standards and the literatures combining accurate mass measurement, a total of 91 compounds from XEQJ were unambiguously identified or tentatively characterized, which was valuable for the quality control of XEQJ.

2. Experimental

2.1 Materials and chemicals

Eight reference standards, including rutin, luteoloside, isoquercitrin, lonicerin, calceolarioside B, isoacteoside, apigetrin, and indirubin, were obtained from the National Institutes for Food and Drug Control (Beijing, China). Sixteen reference standards of neochlorogenic acid (3-CQA), crypt chlorogenic acid (4-CQA), chlorogenic acid (5-CQA), sweroside, secoxyloganin, forsythoside A, forsythoside B, acteoside, kaempferol 3-*O*-rutinoside, isochlorogenic acid B (3,4-DiCQA), isochlorogenic acid A (3,5-DiCQA), isochlorogenic acid C (4,5-DiCQA), tricin-7-*O*-glucoside, diosmetin-7-*O*-glucoside, phillyrin, and macranthoidin A were purchased from Chengdu Biopurify Phytochemcals CO., Ltd. (Sichuan, China). All these reference compounds showed purities no less than 98% by HPLC-DAD analysis. FLJ, FF, CL, IN, CARR, RRP, PH, and (XEQJ) were purchased from Beijing Tongrentang Medicine Corporation Ltd. (Beijing, China). Material of each single herb was authenticated by Dr. Jia−Yu Zhang, Center of Scientific Experiment, Beijing University of Chinese Medicine. The voucher specimen was deposited at Center of Scientific Experiment, Beijing University of Chinese Medicine, China.

HPLC grade acetonitrile, methanol, and formic acid used were purchased from Fisher Scientific (Fisher, Fair Lawn, NJ, USA). De-ionized water used throughout the experiment was purified by Milli-Q system (Millipore, Bedford, MA, USA).

2.2 Sample and standards preparation

For the LC-MS analysis, XEQJ and crude herbal medicines were powdered in a mortal and mill. Approximately 2.0 g pulverized powders were accurately weighed and ultrasonicated with 25 mL of 70% (*v/v*) methanol for 30 min, and then cooled at room temperature. The supernatant solution was filtered and evaporated on water bath at 60℃. The obtained residue was dissolved in 2 mL 70% methanol. Stock solution of the reference standards was prepared in 70% methanol, which could be diluted to prepare the working solution. Prior to injection, the samples were filtered through 0.22 μm membranes. An aliquot of 10 μL of the filtrate was successively injected into the LC-HRMS instrument for analysis.

2.3 HPLC conditions

HPLC analysis was performed on an Agilent series 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump, an on-line degasser, a diode-array detector (DAD), an autosampler, and a column compartment. Samples were separated on a Phenomenex Luna C_{18} column (250 × 4.6 mm i.d., 5 μm) at room temperature. The mobile phase was consisted of 0.1% (*v/v*) formic acid (A) and acetonitrile (B). A gradient program was adopted as follows: 0–26 min, 2–12% B; 26–77 min, 12–26% B; 77–80 min, 26–75% B; 80–88 min, 75–90% B; 88–94 min, 90% B. A 10 min post run time was set to sufficiently equilibrate the column. The flow rate was set at 1.0 mL min^{-1}. The DAD detector scanned from 190 to 400 nm, and the sample were detected at 254 nm.

2.4 Mass spectrometric conditions

A hybrid LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) was connected to the Accela HPLC system equipped with a binary pump and an autosampler (Thermo Scientific, Bremen, Germany) *via* an ESI interface in a post-column splitting ratio of 1: 4. For MS detection, high purity nitrogen (N_2) was used as the sheath gas and auxiliary gas, and ultra-high pure helium (He) as the collision gas. The optimized ESI parameters in the negative ion mode were as follows: capillary temperature, 350℃; sheath gas flow, 30 arb.; auxiliary gas flow, 10 arb.; source voltage, 4.0 kV; capillary voltage, –35 V; tube lens voltage, –110 V. The analysis was operated in both negative and positive ion mode with a mass range of *m/z* 100–1500. In the positive ion mode, the capillary voltage was 25 V; tube lens voltage was 110 V; other parameters were same as those of negative ion mode. MS full scan was detected by High-resolution (FT) and MS/MS analysis by ion trap dynode. Accurate mass analyses were calibrated according to the manufacturer's guidelines using a standard solution mixture of caffeine, sodium dodecyl sulfate, sodium taurocholate, the tetrapeptide MRFA acetate salt, and Ultramark (Sigma Aldrich, St. Louis, MO, USA). The resolution of the orbitrap mass analyzer was set at 30000. Data-dependent MS^n scanning was used so that the two most abundant ions in each scan were selected and subjected to tandem mass spectrometry (MS^n, n=3). The isolation width was 2 amu, and the normalized collision energy (CE) was 35% for all compounds. CID was conducted in LTQ with an activation q of 0.25 and activation time of 30 ms. MS scan functions and HPLC solvent gradients were controlled by the Xcalibur data system (Thermo Scientific), and all the data were collected and processed by using Xcalibur 2.1 software (Thermo Scientific).

3 Result and discussion

3.1 Optimization of analytical conditions

In order to reveal as many chemical constituents of XEQJ as possible and achieve adequate structural information of the chemical compounds with different structure types, both negative and positive modes were examined in this experiment. Generally, phenylethanoid glycosides, phenolic acids, flavonoids, lignans, and iridoid glycosides were readily to be ionized and fragmented in the negative ion mode, while alkaloids and saponins especially C_{21} steroidal saponins preferred the positive ion mode.

3.2 HPLC/ESI-MS^n analysis of XEQJ

Fig. 1 showed the total ion chromatograms (TIC) of XEQJ and reference standards. The certain compounds in complex XEQJ matrices could be rapidly screened from complex matrices by extracted ion chromatograms (EIC) with a determined narrow mass window, and then be unequivocally identified by comparison the fragmentation patterns, retention time, chromatographic behavior, and MS^n data with those of reference standards. The diagnostic ions representing a certain substructure or substituent group were then deduced, and the fragmentation mechanisms were also proposed. Diagnostic ions and fragmentation mechanisms from reference compounds were of great importance for screening and identifying unknown compounds. For those unknown constituents, we first determined the molecular formula based on the accurate mass obtained from HRMS, and then the diagnostic ions deduced from reference standards were adopted to rapidly locate the candidates containing such a substructure or substituent group. Combining constituents ever isolated or reported in the literatures, the most possible structure could then be determined from these candidates. Based on the described methods above, a total of 91 compounds (Table 1, Table 2, and Fig. 2) were unambiguously identified or tentatively characterized from XEQJ, 24 of which were confirmed by their reference standards. Moreover, 2 of them were potential new compounds from FF. These compounds included 33 phenylethanoid glycosides, 13 phenolic acids, 11 flavonoids, 10 alkaloids, 9 ligans, 9 iridoid glycosides, and 6 saponins. The component herb from which each compound was derived was confirmed by individually analyzing seven herbs of XEQJ except Gypsum Fibrosum using the same HPLC/ESI/-LTQ-Orbitrap MS method.

3.2.1 Structural characterization and identification of phenylethanoid glycosides.

A total of 33 phenylethanoid glycosides were screened, among which 25 were from FF, 6 from PH, and 5 from RRP. Five phenylethanoid glycosides were unambiguously identified as forsythoside B (**Pg17**), forsythoside A (**Pg21**), acteoside (**Pg22**), calceolarioside B (**Pg26**), and isoacteoside (**Pg28**) by comparison with their respective reference compounds. Depending on whether the hydrogen atom in the β position was substituted, these compounds could be classified into two different types.[18]

Type I (the β position was not substituted)

The ESI-MS spectrum of forsythoside B (**Pg17**) produced an $[M - H]^-$ ion at *m/z* 755.2387 ($C_{34}H_{43}O_{19}$). Its fragmentation was triggered by initial loss of the caffeoyl unit to yield a prominent ion at *m/z* 593, and the losses of apiose and rhamnose or both were observed with the formation of ions at *m/z* 461 $[593 - 132]^-$, 447 $[593 - 146]^-$, and 315 $[593 - 132 - 146]^-$. Other minor ions at *m/z* 443, 429, and 297 corresponding to successive loss of water from ions at *m/z* 461, 447, and 315, were also respectively detected. And another minor ion at *m/z* 179 corresponding to $[\text{caffeic acid} - H]^-$ was also observed. The proposed fragmentation pathway of forsythoside B was shown in Fig. 3. The ESI-MS/MS spectra of forsythoside A (**Pg21**) were similar to those of **Pg17**. Its $[M - H]^-$ ion yielded a lot of fragment ions at *m/z* 477, 461, 315, and 135, owing to the neutral loss of rhamnose and successive losses of the caffeoyl residue, rhamnose, hexose, and water from the $[M - H]^-$ ion at *m/z* 623.1957 ($C_{29}H_{36}O_{15}$). For acteoside (**Pg22**) and isoacteoside (**Pg28**), which were isomers of **Pg21,** had the same fragment ions with **Pg21**. Calceolarioside B (**Pg26**) gave quasi-molecular ion at *m/z* 477.1397 ($C_{23}H_{26}O_{11}$), and two abundant fragment ions at *m/z* 161 and 315 corresponding to $[\text{glc} - H - H_2O]^-$ and $[M - H - \text{caffeoyl}]^-$. The above fragmentation behavior was in accordance with the characteristic fragmentation patterns of phenylethanoid glycosides previously reported,[19−21] and valuable in screening and deducing uncertain compounds belonging to the same class.

Pg18 yielded the identical $[M - H]^-$ ion at *m/z* 755.2389 ($C_{34}H_{43}O_{19}$) to **Pg17**, and fragmentation in the same way, it was thus assigned as an isomers of forsythoside B.

Pg13 and **Pg19** had identical molecular ions and fragmentation ions to **Pg21**, thus they were plausibly identified as isomers of forsythoside A. Considering compounds isolated from FF, forsythoside H and I were the most possible candidates. Herein, a parameter of *C*log*P* was adopted to determine the elution order. *C*log*P* is the calculated value of log*P* (*n*-octanol/water partition coefficient), which is predicated by the software ChemBioDraw Version 11.0 (Cambridge-Soft, Cambridge, MA, USA) based on theoretical calculations. Generally, the compound with larger *C*log*P* value would yield a larger retention time on reverse-phase HPLC.[22] Thus, **Pg13** and **Pg19** were tentatively characterized as forsythoside H (*C*log*P*: –0.9526) and I (*C*log*P*: –0.8902), respectively.[23]

Pg8 generated $[M-H]^-$ ion at *m/z* 785.2494 ($C_{35glcglc}H_{45}O_{20}$), 162 Da more than **Pg21**. MS/MS of the $[M-H]^-$ ion yielded a product ion at *m/z* 623 and have similar diagnostic ions to **Pg21** (Table 1), in addition, it was from RRP. Thus, this peak was characterized as echinacoside, a known compound isolated from RRP.[24] **Pg10** showed $[M-H]^-$ ion at *m/z* 639.1920 ($C_{29}H_{35}O_{16}$), 16 Da higher than **Pg21**, corresponding 16 Da higher than the base peak of **Pg21** at *m/z* 477 $[461+16]^-$ in MS^2 spectrum and yielded very similar MS^3 data to **Pg21** (Table 1), indicating that rhamnose was substituted by hexose, similarly, **Pg3** had same relationship with **Pg8**. Thus, **Pg10** and **Pg3** were potential new compounds from FF.

The spectra of **Pg11**, **Pg14,** and **Pg16** were extremely similar with that of **Pg21**, except that a pentose rather than a rhamnose loss was observed. These peaks were thus identified as calceolarioside C or other unknown isomer.[18] Similarly, **Pg23** was identified as forsythoside G, a known compound isolated from FF.[18]

Pg12 showed molecular ion at *m/z* 799.2648 ($C_{36}H_{47}O_{20}$). Whose MS^3 spectrum was identical to MS^2 spectrum of **Pg21** and it generated a base peak at *m/z* 623 formed by loss of 176 Da from *m/z* 799. It was revealed that a methylation caffeoyl unit (176 Da) was presented. **Pg24** exhibited

[M − H]⁻ ion at *m/z* 813.2802 ($C_{37}H_{49}O_{20}$), 14 Da higher than **Pg12**, corresponding to an fragmentation ion at *m/z* 329 was 14 Da higher than *m/z* 315 of **Pg12**, indicating a methoxyl group might lay in phenylethyl moiety. Thus, **Pg12** and **Pg24** were tentatively characterized as jionoside A and jionoside B, two known compounds isolated from RRP.[25]

Pg29 and **Pg31** both exhibited [M − H]⁻ ions at *m/z* 637, 14 Da higher than **Pg21**, but they had same fragmentation pathway as **Pg21**, indicating that the caffeoyl unit (162 Da) in **Pg21** was replaced by methylation caffeoyl unit (176 Da). Thus, leucosceptoside A came from PH was considered to be one appropriate candidate for **Pg29** or **Pg31**.[26] Similarly, **Pg32** and **Pg33** gave [M − H]⁻ ions at *m/z* 651, which was 28 Da higher than **Pg21**, indicating the presence of two methoxyl groups. The formation of ions at *m/z* 475 and 329, 14 Da higher than *m/z* 461 and 315 of **Pg21**, revealed one methoxyl group lay in caffeoyl unit, another one might lie in phenylethyl moiety. Cistanoside D derived from PH was considered to be the one appropriate candidate for **Pg32** and **Pg33**.[27]

Pg4 gave an [M − H]⁻ ion at *m/z* 299.1129 ($C_{14}H_{19}O_7$), 324 Da less than **Pg21**, and had the same fragmentation pathway with **Pg21**, indicating the absence of caffeoyl, rhamnose and hydroxyl. Therefore, salidroside derived from FF was considered to be appropriate candidate for **Pg4**.[28] Similarly, **Pg5** was assigned as forsythoside E.[23]

Pg15 and **Pg20** produced identical molecular ions to **Pg26**, and fragmented in the same way. They were thus assigned as calceolarioside B isomers. According to the literature, calceolarioside A derived from FF was considered to be the most suitable candidate for **Pg15** and **Pg20**.[29]

Type II (the β position was substituted)

In contrast to **Pg21** (forsythoside A), suspensaside A showed a significantly different fragmentation pattern which resulted from the etherification of the C-2 position of glucose and the β position of phenylethyl group. On one hand, similar to type I, its fragmentation was initially triggered by loss of caffeoyl moiety to yield an abundant ion at *m/z* 459, accompanied by the sequential loss of water to produce an ion at *m/z* 441. The MS^3 spectrum of *m/z* 459 gave a base peak at *m/z* 151, corresponding to losses of rhamnose (146 Da) and hexose (162 Da). On the other hand, because of a special ether ring, neutral loss of 134 Da was easily observed and yield a product ion at *m/z* 487, also produced an ion at *m/z* 469, corresponding to the sequential loss of water. Other minor ions such as *m/z* 427 $[487 - 60]^-$, and 397 $[487 - 90]^-$, were deduced to stem from hexose unit.[30–32] The ion at *m/z* 469 was subjected to fragment to give product ions at *m/z* 409, 179, and 161, corresponding to the cleavage of hexose, $[\text{caffeic acid} - H]^-$, $[\text{glc} - H - H_2O]^-$. According to the fragmentation information described above, **Pg25**, **Pg27**, **Pg30** were tentatively characterized as isomer of suspensaside A, a known compound isolated from FF.[18]

The hydrogen atom in the β position of (R)-suspensaside and (S)-suspensaside were substituted by hydroxyl group, thus fragmentation observed in MS^2 spectra of the $[M - H]^-$ was triggered by initial loss of water to generate a significant ion at *m/z* 621, during the following fragmentation, ions at *m/z* 487, 469, 459, 441, 179 were observed, which was similar to suspensaside A, indicating that the ion at *m/z* 621 might have the same structure as suspensaside A.[18] The fragmentation behavior of **Pg6**, **Pg7** and **Pg9** were consistent with described above. Combining the elution order reported before and *C*log*P* value, **Pg6** (*C*log*P*: −2.0278), **Pg7** (*C*log*P*: −2.0278) and **Pg9** (*C*log*P*: −1.9756) were reasonably speculated as (R)-suspensaside, (S)-suspensaside and β-hydroxyacteoside, respectively.[18] The proposed fragmentation pathway of this type of phenylethanoid glycosides was shown in Fig. 4.

Pg1 and **Pg2** both gave $[M - H]^-$ ions at *m/z* 477, 162 Da less than (R)-suspensaside or (S)-suspensaside, and these fragmentation patterns were identical to suspensaside A, indicating absence of caffeoyl unit compared to (R)-suspensaside or (S)-suspensaside. Thus, forsythoside D derived from FF was considered to be the one appropriate candidate for **Pg1** and **Pg2**.[33]

3.2.2 Structural characterization and identification of phenolic acids

The structure of phenolic compounds were esters formed between quinic acid and one to four residues of certain *trans*-cinnamic acids, commonly including caffeic, *p*-coumaric, ferulic, and sinapic. A total of 13 phenolic acids were screened from XEQJ, which were all from FLJ. Their characteristic fragmentation pathways were first characterized by the loss of one or two cinnamic acid moiety and successively by dehydration.[34] Besides, a quinic acid moiety at *m/z* 191, a dehydrated quinic acid moiety at *m/z* 173, a cinnamic acid moiety at *m/z* 179 [caffeic acid – H]$^-$, 193 [ferulic acid – H]$^-$, and 163 [coumaric acid – H]$^-$, a dehydrated caffeic acid moiety at *m/z* 161 and a decarboxylated caffeic acid moiety at *m/z* 135 were also observed in their ESI-MS experiment. Peaks **Pa2**, **Pa4**, **Pa5**, **Pa9**, **Pa10**, and **Pa11** could be unambiguously identified as 3-CQA, 5-CQA, 4-CQA, 3,4-DiCQA, 3,5-DiCQA, and 4,5-DiCQA by comparison with reference compounds. Additionally, **Pa2**, **Pa4,** and **Pa5** were a group of CQA isomers, while **Pa9**, **Pa10** and **Pa11** were a group of DiCQA. We could speculate the substitution position of caffeoyl according to the kind and relative intensity of base peak in their ESI-MS^n spectra.[35] For peak **Pa3**, **Pa6,** and **Pa7** all released the $[M - H]^-$ ions at *m/z* 377 corresponding to *p*-coumaroylquinic acid (*p*-CoQA). In their MS^2 spectra, the base peak was remarkably different. Generally, esterification at positions 3, 4, 5, or 1 of quinic acid moiety produced base peaks at *m/z* 163, 173, and 191, respectively.[36] However, considering the polarity of 5-*p*-CoQA is weaker than that of 3-*p*-CoQA. Thus, **Pa3**, **Pa6** and **Pa7** were identified as 3-*p*-CoQA, 5-*p*-CoQA, and 4-*p*-CoQA, respectively. Furthermore, **Pa8,** a feruloylquinic acid (FQA) that generated ESI–MS^2 base peak at *m/z* 191 was detected. Hence, it was tentatively characterized to be 5-FQA[36]. Peak **Pa1** produced deprotonated molecular ion at *m/z* 191 and fragment ions at *m/z* 173 $[M-H-H_2O]^-$, and 127 $[M-H-H_2O-H_2O-CO]^-$. So, it was characterized as quinic acid. For peak **Pa12** and **Pa13**, both exhibited $[M-H]^-$ ions at *m/z* 529 and fragment ions at *m/z* 367, 14 Da higher than *m/z* 353 in DiCQA. Thus, they were tentatively assigned as methylated dicaffeoylquinic acid.

3.2.3 Structural characterization and identification of flavonoids.

A total of 11 compounds were screened and identified as flavonoids from XEQJ, among which, 10 were from FLJ, 3 from FF, and 2 from PH. 8 flavonoids were unambiguously identified as rutin (**F1**), quercetin-3-*O*-glucoside (**F2**), luteolin-7-*O*-glucoside (**F3**), lonicerin (**F4**), kaempferol 3-*O*-rutinoside (**F5**), apigetrin (**F7**), diosmetin-7-*O*- glucoside (**F10**), and tricin-7-*O*- glucoside (**F11**), by comparison of retention time and mass spectra with those of reference substances.

In ESI-MS experiments, the glycosidic bond of *O*-glycosides in flavonoids was easily cleaved in the negative ion mode to produce aglycone ion (Y_0^-) of $[M-H-162]^-$ and $[M-H-308]^-$ corresponding to loss of hexose sugar and rutinose unit. Sometimes, $[Y_0^- - H]^-$ occurred in the MS spectrum, especially flavonol glycosides. Dehydration, successive loss of CO or loss of CO_2 due to the presence of phenolic hydroxyl groups and a ketone group, Retro-Diels-Alder (RDA) fragmentation, C ring fragmentation and loss of CHO· were the most possible fragmentation pathways for flavonoids.[37–39]

Here we take **F6** as example to illustrate the fragmentation pathways of flavonoids lacking reference standards. F6 produced a high intensity $[M-H]^-$ ion at *m/z* 477.1036 ($C_{22}H_{21}O_{12}$) and

Y_0^- ion at *m/z* 315 by loss of 162 Da from the $[M - H]^-$ ion. For the further cleavage of *m/z* 315, it produced other characteristic fragment ions. Such as $[Y_0 - CH_3]^-$, $[Y_0 - H]^-$, $[Y_0 - H - CH_3]^-$, $[Y_0 - H - CH_3 - CO]^-$, and $^{1,3}A^-$ at *m/z* 300, 314, 299, 271, and 151, respectively. So the aglycone of F6 was plausibly assigned to be isorhamnetin. Thus, **F6** was tentatively identified as isorhamnetin-*O*-hexoside. Similarly, **F8**, a disaccharide conjugate of chrysoeirol, was tentatively identified as chrysoeirol-7-*O*-neohesperidoside and **F9**, a disaccharide conjugate of tricin, was tentatively identified as tricin-7-*O*-neohesperidoside, these compounds were previously isolated from FLJ.[40]

3.2.4 Structural characterization and identification of alkaloids.

A total of 10 compounds were characterized to alkaloids, 9 of which were from CL and 1 from IN.

A10 was certainly assigned to be indirubin by comparison of retention time and mass spectra with those of reference compound. The rest of 9 compounds, come from CL, could be sorted into four types based on their different skeleton.[41]

Type I Cinnamic acid amides

A1, **A2,** and **A5** were tentatively identified as Kukoamine B, Dihydro-*N*-caffeoyltyramine and Trans-*N*-feruloyltyramine by comparison to the fragmentation ions reported in literature.[41] **A5** gave an $[M + H]^+$ ion at *m/z* 314.1380 ($C_{18}H_{20}NO_4$). CID of $[M + H]^+$ was preferential to cleave the amide bond to eliminate the tyramine moiety (137 Da) and produced base ion at *m/z* 177, which ascribed to a feruloyl group. Further fragmentation was consecutively loss of CH_3OH and CO corresponding to *m/z* 145 and 117, respectively. Compared to structure **A5**, the double bond in caffeoyl moiety was reduced in **A2**, which resulted in quite different fragmentation behavior in MS^n experiment. Two predominant ions at *m/z* 138 and 121 were observed in the MS^2 spectrum. It was speculated that after the cleavage of amide bond, electrons were transferred and eliminated dihydro caffeoyltyramine (164 Da) to generate the tyramine ion (*m/z* 138), which subsequently lost NH_3 to produce the ion of *m/z* 121. **A1** was tentatively identified as Kukoamine B by its MS^n data shown in Table 1.

Type II Lignanamides

Lignanamides **A3** and **A7** contained dihydrogen naphthalene skeleton, which affected the fragmentation pattern considerably. For **A7**, it gave $[M + H – tyramide]^+$ ion at *m/z* 504 and other three characteristic ions at *m/z* 231, 394, 339. In pathway I, elimination of 3,4-dihydroxy-N-(4-hydroxyphenethyl) benzamide (273 Da) moiety from $[M + H – tyramide]^+$ yielded diagnostic ion of *m/z* 231. In pathway II, a characteristic ions at *m/z* 394 were formed by the neutral loss of a pyrocatechol unit (110 Da) from $[M + H – tyramide]^+$. In pathway III, the other tyramide was lost from the ion of *m/z* 476 $[M + H – tyramide – CO]^+$ to generate a stable ion of *m/z* 339. The fragmentation behavior observed above was consisted with previous reports. Thus, **A7** was tentatively assigned as 7-hydroxy-1-(3, 4-dihydroxy)-N^2, N^3-bis (4-hydroxyphenethyl)-6, 8-dimethoxy-1, 2-dihydronaphthalene-2, 3-dicarboxamide. Similarly, **A3** was tentatively assigned as (1,2-trans)-N^3-(4-acetamidobutyl)-1-(3,4-dihydroxyphenyl)-7-hydroxy-N^2-(4-hydroxyphenethyl) -6, 8-dimethoxy-1, 2-dihydro-naphthalene-2, 3-dicarboxamide.[41]

Type III Neolignanamides

The basic skeleton of **A8** contains a special bond between the two cinnamoyltyramine derivatives. Accordingly, the main specific fragmentation of it was continuous losses of two tyramide moieties, which produced the ions at *m/z* 506 and 369. Elimination of CO and plus 2H to yield *m/z* 343 from the ion at *m/z* 369. Subsequent consecutive losses of H_2O and MeOH from the ion at *m/z* 343 to produced other two ions at *m/z* 325 and 293. Hence, this compound was assigned as (E)-2-(4, 5-dihydroxy-2-{3-[(4-hydroxyphenethyl)amino]-3-oxopropyl}phenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-*N*-(4-hydroxyphenethyl) acrylamide, which had been isolated from CL.[41]

Type IIII cyclic peptides

The fragmentation of cyclic peptides (compounds **A4**, **A6**, **A9**) mainly occurred in the side chain. CID of **A4** yielded molecular ion at *m/z* 874.3707 ($C_{42}H_{52}N_9O_{12}$) and the dehydration ion at *m/z* 856, which subsequently lost an amino acid fragment of pyroGlu-Pro-Tyr (388 Da) to form the ion at *m/z* 468. Further fragmentation of *m/z* 468 was also observed. Continuous losses of HCOOH or CO_2 produced ions at *m/z* 422 and 424 from *m/z* 468. In addition, *m/z* 486 was origin from *m/z* 874 by loss of the side chain (388 Da), *m/z* 503 was formed by the cleavage of the amide bond to eliminate a molecular (371 Da) from $[M + H]^+$ ion at *m/z* 874. **A6**, **A9** had identical fragmentation pathway. According to the literature, **A4**, **A6** and **A9** were tentatively characterized as Lyciumin A, Lyciumin B and Lyciumin C.[42]

3.2.5 Structural characterization and identification of ligans

A total of 9 ligans were screened from XEQJ. And all of them were from FF, **L9** was unambiguously identified as phillyrin by comparison with reference compound.

The ligans could be classified into two types according to their structure skeleton: the furoruran type (I) and the 2,3-dibenzyl butyrolactone type (II).[18]

Type I

(+)-1-hydroxylpinoresinol, underwent the characteristic cleavage of the tetrahydrofuran ring to produce the ion at *m/z* 343, followed by cleavage of another tetrahydrofuran ring to yielded m/z 313, MS^n of *m/z* 313 showed an abundant ion at *m/z* 298, owing to loss of CH_3 and another minor ion at *m/z* 188, owing to loss of 110 Da from *m/z* 298. Successive loss of 30 Da and loss 110 Da were characteristics of this type of compounds.[43] **L5** generated $[M - H]^-$ ion at *m/z* 373.1285 ($C_{20}H_{21}O_7$) and **L1** yielded $[M - H]^-$ ion at *m/z* 535.1810 ($C_{26}H_{31}O_{12}$), 162 Da higher than **L5**, these fragmentation information were accordance with this characteristics. Hence, **L5** and **L1** were tentatively characterized as (+)-1-hydroxylpinoresinol and (+)-1-hydroxylpinoresinol-*O*-glucoside, respectively.

As reported in the literature,[44] (+)-pinoresinol and (+)-epipinoresinol had some differences with (+)-1-hydroxylpinoresinol in fragmentation pattern, they usually generated an $[M - H - 15]^-$ ion at *m/z* 342, an $[M - H - 30]^-$ ion at *m/z* 327 and $[M - H - 15 - 31]^-$ ion at *m/z* 311, and produced an prominent ion at *m/z* 151 as a result of cleavage of the tetrahydrofuran ring. **L2**, **L3** and **L4** exhibited $[M + COOH]^-$ ions at *m/z* 565 and $[M - H]^-$ ions at *m/z* 519, 162 Da higher than (+)-pinoresinol or (+)-epipinoresinol, they both yielded $[\text{aglycone} - H]^-$ ion at *m/z* 357 in their MS^2 spectrum. MS^n of *m/z* 357 showed the similar fragmentation ions to (+)-pinoresinol or (+)-epipinoresinol, combing the literature information about their elution behavior, they were speculated as (+)-pinoresinol-*O*-glucoside, (+)-epipinoresinol-4"-*O*-glucoside and (+)-epipinoresinol-4'-*O*-glucoside, respectively.

L8 showed $[M - H]^-$ ion at *m/z* 371.1488 ($C_{21}H_{23}O_6$), 14 Da higher than (+)-pinoresinol or (+)-epipinoresinol, also exhibited similar fragmentation pathway and produced ions at *m/z* 356, 341 and 326, indicating that one more methyl was existed in **L8** compared with (+)-pinoresinol or (+)-epipinoresinol. Therefore, **L8** was identified as Phillygenin, tentatively.

L9 gave $[M+COOH]^-$ ion at *m/z* 579.2063 ($C_{28}H_{35}O_{13}$) and $[M - H]^-$ ion at *m/z* 533, yield similar fragmentation ions to **L8** (Table 1), thus, it was unambiguously identified as phillyrin with reference to the standard. **L7** possessed identical fragmentation ions and fragmentation pattern to **L9**. According to the literature, it was easily considered to be an isomer of phillyrin, thus, it was identified as (+)-pinoresinol monomethyl ether *O*-glucoside.[18]

Type II

Matairesinol, a compound with 2,3-dibenzyl butyrolactone in FS, produced $[M-H]^-$ ion at *m/z* 357. The MS/MS spectrum of the ion at *m/z* 357 gave a significant product ion at *m/z* 313, which revealed lactone ring in the structure. Successive elimination of CH_3 from the precursor ion at *m/z* 313, corresponding *m/z* 298 and 283 further confirmed the presence of two methoxyl groups. The ion at *m/z* 161 was also observed owing to cleavage of the benzyl group.[18] Briefly, compound of this type could be rapidly recognized by loss of CO_2 and cleavage of the benzyl group. **L6** gave the $[M-H]^-$ ion at *m/z* 519, 162 Da higher than matairesinol, it had identical fragmentation pathway to matairesinol, thus, it was rapidly characterized as matairesinoside.[45]

3.2.6 Structural characterization and identification of iridoid glycosides.

Iridoid glycosides generally contain a glucose moiety attached to the C-1 position in the pyran ring. A total of 9 compounds were screened and identified as iridoid glycosides with their retention time and MS data shown in Table 1. Among them, 8 were from FLJ, 2 were from RRP and 1 from FF. Compounds of this category commonly eliminate a glucose unit (162 Da) in the pyran ring and subsequent losses of H_2O, CO_2 and CO. Neutral elimination of CH_3OH was also generally observed in methoxylated iridoid glycosides. These observations were consistent with previous study.[46–47]

Peak **I7** and **I8** were unambiguously characterized to be sweroside and secoxyloganin, respectively, by comparison with reference compounds. **I7** generated a predominant $[M+COOH]^-$

at *m/z* 403.1236 ($C_{17}H_{23}O_{17}$) and $[M - H]^-$ at *m/z* 357. An obvious fragment ion $[M - H - Glc]^-$ at *m/z* 195 was characterized by loss of neutral glucose unit. Successive losses CO_2 and CO from $[M - H - Glc]^-$ yielded another two ions at *m/z* 151 and 167. Another minor ion at *m/z* 125 originated from RDA cleavage in the aglycone moiety.

I1 produced $[M - H]^-$ ion at *m/z* 523.1656 ($C_{21}H_{31}O_{15}$) and also generated other ions such as $[M - H - Glc]^-$, $[M - H - Glc - Glc]^-$, $[M - H - H_2O]^-$, $[M - H - H_2O - H_2O]^-$, $[M - H - H_2O - Glc]^-$, $[M - H - H_2O - Glc - Glc]^-$ corresponding to *m/z* 361, 199, 505, 487, 343, 181, respectively. According to these fragmentation ions, **I1** was tentatively identified as rehmannioside A or its other isomer presented in RRP. **I2** and **I4**, two isomers, yielded identical $[M - H]^-$ ions at *m/z* 375, according to their ESI-MS data in Table 1, lognin acid and 8-*epi*-loganin acid were suitable candidates for **I2** and **I4**.[37] **I5**, which generated $[M - H]^-$ ion at *m/z* 389.1078 ($C_{16}H_{21}O_{11}$) and other fragmentation ions at *m/z* 345, 183, 165 formed by $[M - H - CO_2]^-$, $[M - H - CO_2 - Glc]^-$, $[M - H - CO_2 - Glc - H_2O]^-$, was tentatively characterized as secologanoside.[48] **I3** and **I6**, both exhibited deprotonated molecular ions at *m/z* 373, 16 Da (O) less than that of **I5**, and other fragmentation ions like $[M - H - Glc]^-$ at *m/z* 211, $[M - H - Glc - CO_2]^-$ at *m/z* 167, $[M - H - Glc - CO_2 - H_2O]^-$ at *m/z* 149, $[M - H - Glc - CO_2 - H_2O - C_2H_2]^-$ at *m/z* 123 were observed in their MS spetrum. Thus, they were characterized to be secologanic acid.[49] **I9** yielded deprotonated molecular ion at *m/z* 417.1397 ($C_{18}H_{25}O_{11}$), 28 Da (2 CH_2) higher than that of **I5**, also produced other characteristic ions as $[M - H - Glc - H_2O]^-$ at *m/z* 237, $[M - H - CH_3OH]^-$ at *m/z* 385, $[M - H - CH_3OH - CO_2]^-$ at *m/z* 341. Thus, **I9** could tentatively assigned as dimethyl-secologanoside.[37]

3.2.7 Structural characterization and identification of saponins.

A saponin molecule consists of an aglycone and sugar units. In this study, a total of 6 saponins were characterized as saponins, in which, 1 compound, come from FLJ, was triterpenoid saponin and other 5 compounds, come from CARR, were all C_{21} steroidal saponins.

S1 was unambiguously identified as macranthoidin A by comparison with reference standard. Under negative ion mode, **S1** yielded deprotonated molecular ions at *m/z* 1235, successive loss glucose and rhamnose gave other ions at *m/z* 1073 $[M - H - Glc]^-$, 927 $[M - H - Glc - Rha]^-$, 911 $[M - H - Glc - Glc]^-$.

The MS^n spectra in positive ion mode of C_{21} steroidal saponins provided a wealth of structural information. This type of saponins tended to generated $[M + Na]^+$, and showed abundant ion for the loss of HCOOH (46 Da) explained by a McLaffery rearrangement from $[M + Na]^+$.[50] Fragmentation ions formed by losses of a series of sugar residues and oligosaccharide plus sodium were also observed in their spectrum. The fragmentation pattern observed was consistent with previous publication.[50] Compounds **S2**, **S3**, **S4**, **S5** and **S6** were both C_{21} steroidal saponins, and they were tentatively assigned as glaucogenin C-*O*-*β*-D-thevetopyranoside, cynaversicoside F, cynaversicoside A, atratoglaucoside A and glaucoside C by comparion with the literatures.[50–52] Here, we take **S6** (glaucoside C) as example to elaborate the fragmentation pathways of C_{21} steroidal saponins. **S6** produced $[M + Na]^+$ at *m/z* 817.3957 ($C_{41}H_{62}O_{15}Na$) and gave a high intensity ion $[M + Na - HCOOH]^+$ at *m/z* 771. Ions such as *m/z* 627, 497 and 353 were corresponding to the successive losses of cymarose, digitoxose and cymaroce from $[M + Na - HCOOH]^+$. The other two ions at *m/z* 291, 441 were observed owing to $[cym + dgt + Na]^+$ and $[cym + dgt + cym + Na]^+$. Besides, continuous losses of cymarose and digitoxose from $[M + Na]^+$ were also observed with the formation of ions at *m/z* 673 and 543.

All the other C_{21} steroidal saponins showed the similar fragmentation pattern as described above for S6, and their structures were elucidated by analyzing their tandem mass spectra.[53]

4 Conclusion

Our study took the advantage of the LTQ-Orbitrap mass spectrometry system and reported the identification of 91 compounds with multiple structure types including phenylethanoid glycosides, phenolic acids, flavonoids, alkaloids, ligans, iridoid glycosides and saponins. The results clearly elucidated the potential fragmentation pathway of the multi-groups of constituents in XEQJ and this method has also been shown to be an excellent tool for systematic characterization of those in XEQJ. This research not only provides abundant information for the identification and better understanding of the chemical compounds in XEQJ, but also benefits further quality control of XEQJ. Moreover, this study sets a good example for the rapid identification of complex chemical constituents in TCM and opens perspectives for similar studies on other Chinese herbal preparations.

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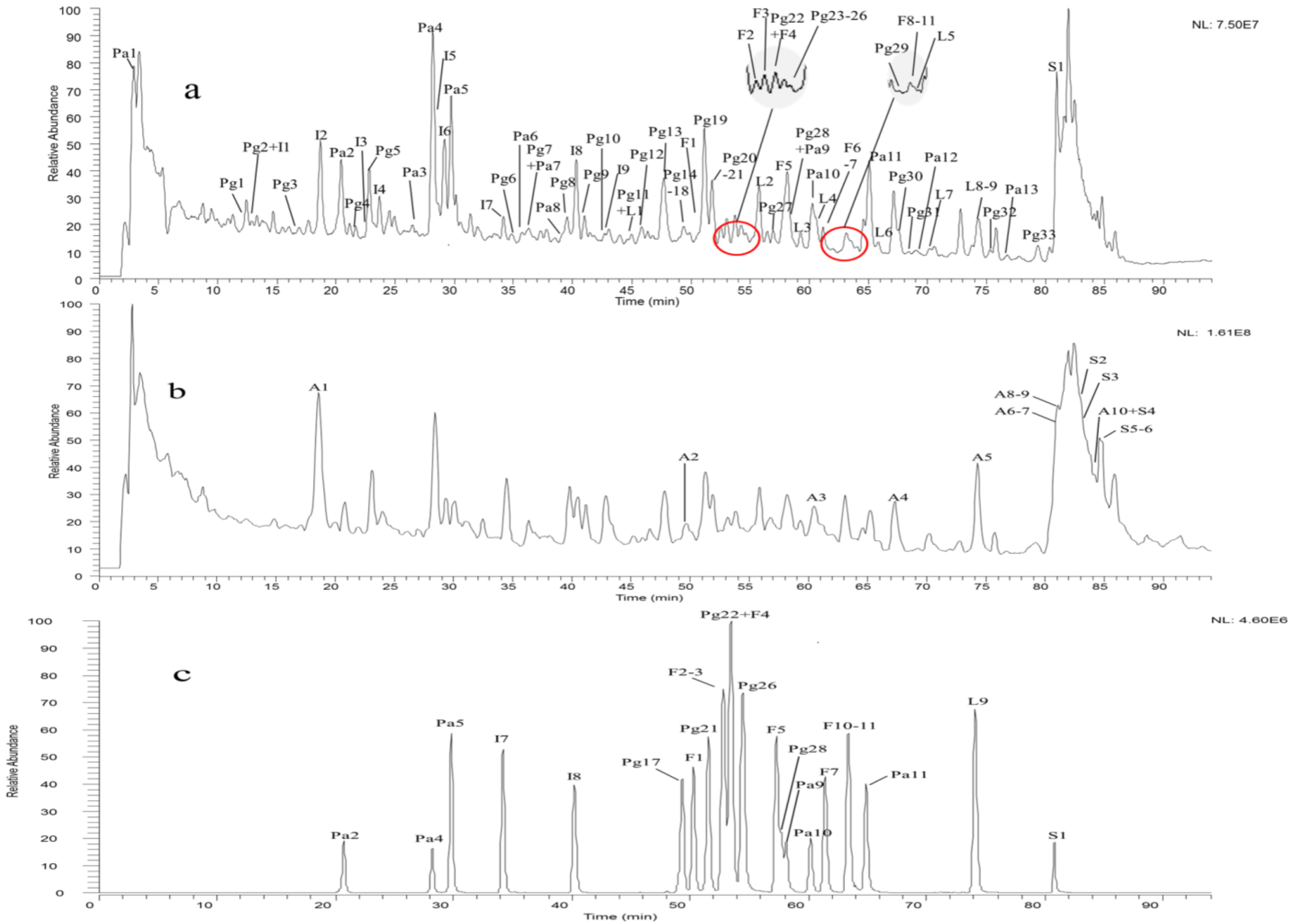
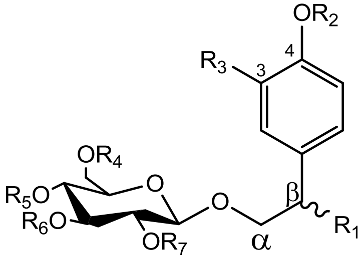
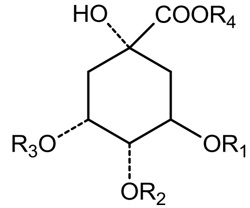


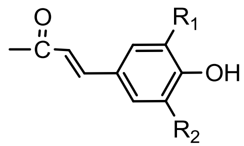
Fig. 1. ESI-MS total ion chromatograms of XEQJ and reference standards: (a) XEQJ scanned in negative ion mode, (b) XEQJ scanned in positive ion mode and (c) reference standards scanned in negative ion mode.



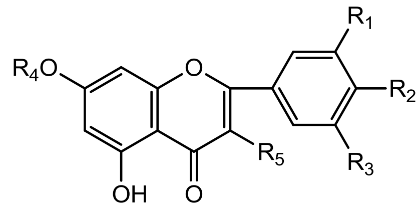
Pg1 or Pg2: R1= OH, R2=H, R3=OH, R4=-rha, R5=H, R6=H, R7=H
Pg3: R1= H, R2=H, R3=OH, R4=-glu, R5=H, R6=H, R7=H
Pg4: R1= H, R2=H, R3=H, R4=H, R5=H, R6=H, R7=H
Pg5: R1= H, R2=H, R3=OH, R4=-rha, R5=H, R6=H, R7=H
Pg6: R1= OH, R2=H, R3=OH, R4=-rha, R5=caffeoyl, R6=H, R7=H
Pg7: R1= OH, R2=H, R3=OH, R4=-rha, R5=caffeoyl, R6=H, R7=H
Pg8: R1= H, R2=H, R3=OH, R4=-glu, R5=caffeoyl, R6=-rha, R7=H
Pg9: R1= OH, R2=H, R3=OH, R4=H, R5=caffeoyl, R6=-rha, R7=H
Pg10: R1= H, R2=H, R3=OH, R4=-glu, R5=caffeoyl, R6=H, R7=H
Pg11 or Pg14 or Pg16: R1= H, R2=H, R3=OH, R4=-xyl, R5=caffeoyl, R6=H, R7=H
Pg12: R1= H, R2=H, R3=OH, R4=-glu, R5=methylated caffeoyl, R6=-rha, R7=H
Pg13 : R1= H, R2=H, R3=OH, R4=-rha, R5=H, R6=H, R7=caffeoyl
Pg15 or Pg20 : R1= H, R2=H, R3=OH, R4=H, R5=caffeoyl, R6=H, R7=H
Pg17: R1= H, R2=H, R3=OH, R4=-api, R5=caffeoyl, R6=-rha, R7=H
Pg18: R1= H, R2=H, R3=OH, R4=-api, R5=-rha, R6=caffeoyl, R7=H
Pg19 : R1= H, R2=H, R3=OH, R4=-rha, R5=H, R6= caffeoyl, R7=H
Pg21: R1= H, R2=H, R3=OH, R4=-rha, R5=caffeoyl, R6=H, R7=H
Pg22: R1= H, R2=H, R3=OH, R4=H, R5=caffeoyl, R6=-rha, R7=H
Pg23: R1= H, R2=H, R3=OH, R4=2-O-methylapi, R5=caffeoyl, R6=-rha, R7=H
Pg24: R1= H, R2=CH_3, R3=OH, R4=-glu, R5=methylated caffeoyl, R6=-rha, R7=H
Pg26: R1= H, R2=H, R3=OH, R4=caffeoyl, R5=H, R6=H, R7=H
Pg28: R1= H, R2=H, R3=OH, R4=caffeoyl, R5=H, R6=-rha, R7=H
Pg29 or Pg31: R1= H, R2=H, R3=OH, R4=H, R5=methylated caffeoyl, R6=-rha, R7=H
Pg32 or Pg33: R1= H, R2=H, R3=OCH_3, R4=H, R5=methylated caffeoyl, R6=-rha, R7=H



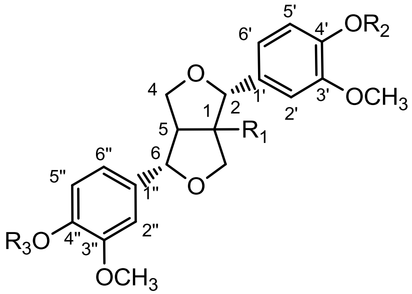
Pa1: R1=H, R2=H, R3=H, R4=H
Pa2: R1= caffeoyl, R2=H, R3=H, R4=H
Pa3: R1= *p*-coumaroyl, R2=H, R3=H, R4=H
Pa4: R1= H, R2=H, R3= caffeoyl, R4=H
Pa5: R1=H, R2= caffeoyl, R3=H, R4=H
Pa6: R1=H, R2=H, R3= *p*-coumaroyl, R4=H
Pa7: R1=H, R2= *p*-coumaroyl, R3=H, R4=H
Pa8: R1=H, R2=H, R3= feruloyl, R4=H
Pa9: R1=caffeoyl, R2= caffeoyl, R3=H, R4=H
Pa10: R1= caffeoyl, R2=H, R3=caffeoyl, R4=H
Pa11: R1= H, R2=caffeoyl, R3=caffeoyl, R4=H
Pa12: R1= caffeoyl, R2=H or caffeoyl, R3=H or caffeoyl, R4=CH_3
Pa13: R1= caffeoyl, R2=H or caffeoyl, R3=H or caffeoyl, R4=CH_3



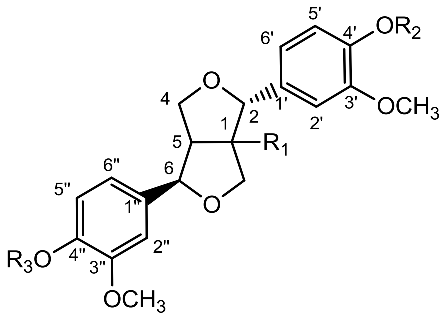
p-coumaroyl: R1=H, R2=H
caffeoyl: R1=OH, R2=H
feruloyl: R1=OCH_3, R2=H



F1: R1=OH, R2=OH, R3=H, R4=H, R5=O-glu$^{6-1}$rha
F2: R1=OH, R2=OH, R3=H, R4=H, R5=O-glu
F3: R1=OH, R2=OH, R3=H, R4=-glu, R5=H
F4: R1=OH, R2=OH, R3=H, R4=-glu$^{2-1}$rha, R5=H
F5: R1=H, R2= OH, R3=H, R4=H, R5=O-glu$^{6-1}$rha
F6: R1=OCH3, R2=OH, R3=H, R4=H, R5=O-glu
F7: R1=H, R2= OH, R3=H, R4=-glu, R5=H
F8: R1=OCH_3, R2=OH, R3=H, R4=O-glu$^{2-1}$rha, R5=H
F9: R1=OCH_3, R2= OH, R3=OCH_3, R4=O-glu$^{2-1}$rha, R5=H
F10: R1=OH, R2=OCH_3, R3=H, R4=-glu, R5=H
F11: R1=OCH_3, R2=OH, R3=OCH_3, R4=-glu, R5=H



L1: R1= OH, R2=H or -glu, R3=H or -glu
L2: R1= H, R2=H or -glu, R3=H or -glu
L5: R1= OH, R2=H, R3=H
L7: R1= H, R2=-glu, R3=CH_3



L3: R1= H, R2=H, R3=-glu
L4: R1= H, R2=-glu, R3=H
L8: R1= H, R2=H, R3=CH_3
L9: R1= H, R2=-glu, R3=CH_3

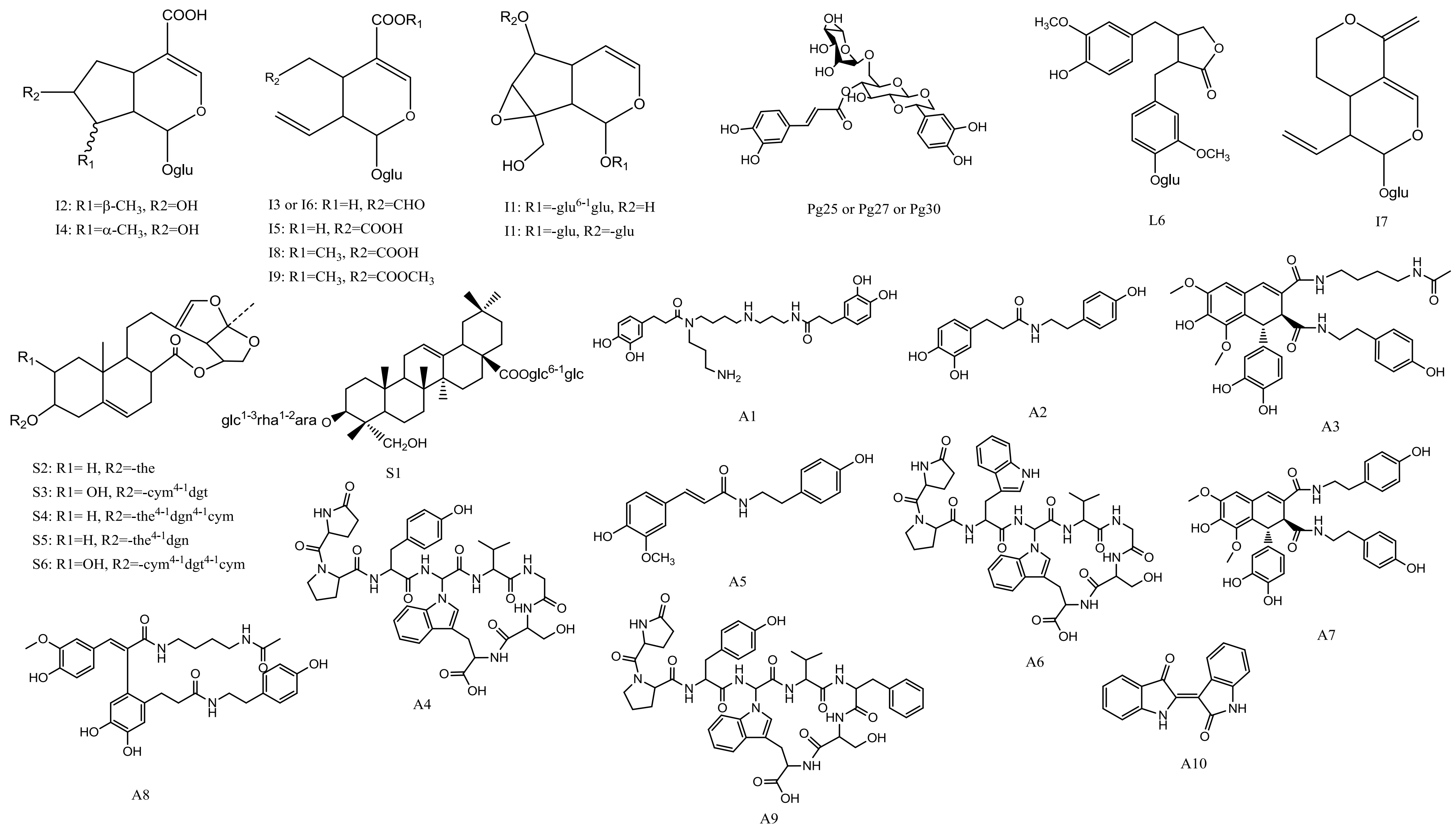


Fig. 2. Chemical structures of compounds identified in XEQJ. glu, glucose; rha, rhamnose; xyl, xylose; api, apiose; the, thevetose; cym, cymarose; dgt, digitoxose; dgn, diginose.

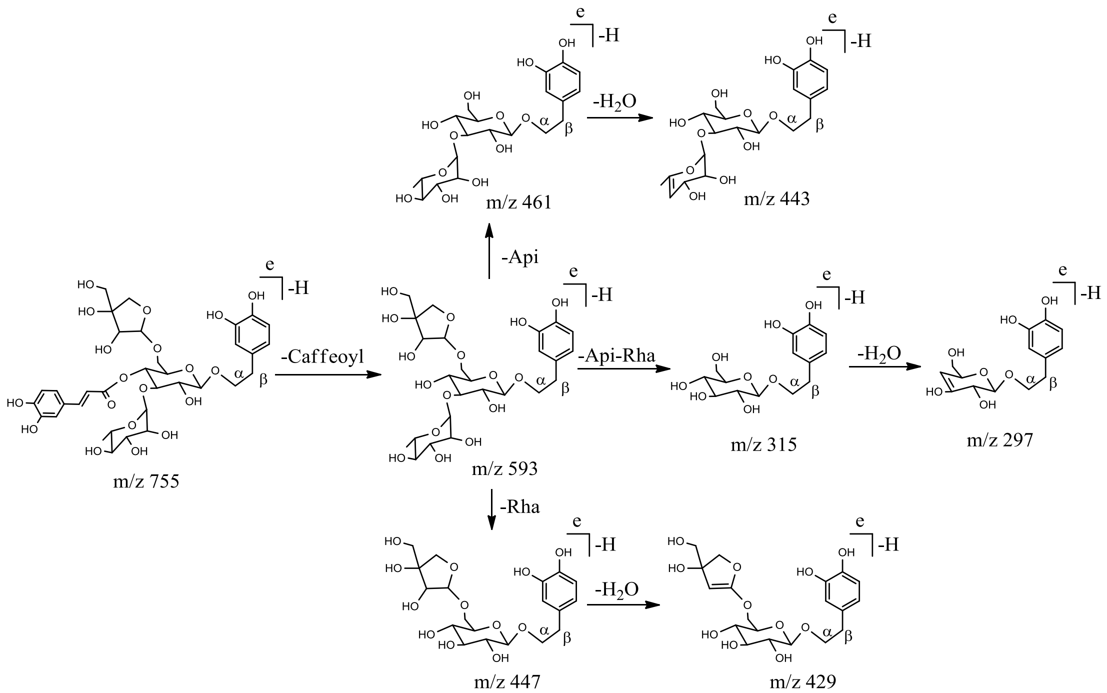


Fig. 3. The proposed fragmentation pathway of forsythoside B.

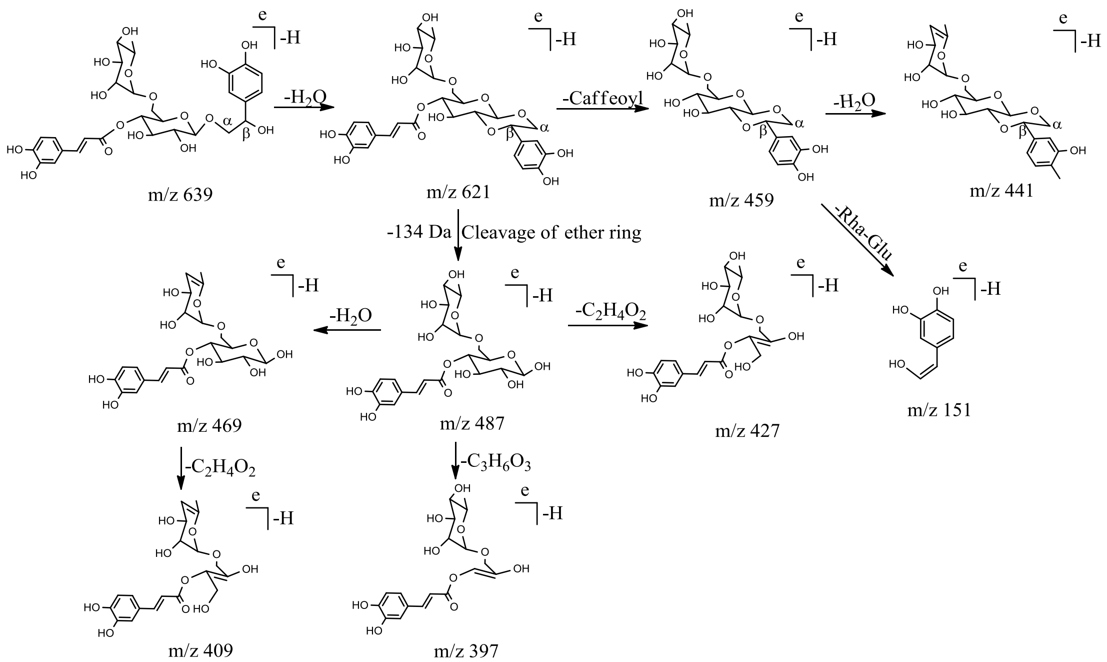


Fig. 4. The proposed fragmentation pathway of suspensaside.

Table 1

Identification of chemical constituents of XEQJ by LTQ-Orbitrap (Negative Ion Mode)

No	t_R (min)	Component Herb	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error (ppm)	Formula $[M-H]^-$	(-)-ESI-MS^n data,P-ion(%)	Identification
Pg1	12.87	FF	477.1603	477.1606	0.781	$C_{20}H_{29}O_{13}$	MS^2[477]:459(100),163(5.9),325(3.5),151(2.6),367(1.5),307(1.4),235(1.3) MS^3[459]:151(100),193(7.5),163(6.7),205 (1.9),325 (1.1),247(0.9)	Forsythoside D or other unknown isomer
Pg2	13.29	FF	477.1603	477.1605	0.530	$C_{20}H_{29}O_{13}$	MS^2[477]:459(100),163(7.5),325(3.8),151(2.6),367(1.5),307(1.4),235(1.4) MS^3[459]:151(100),193(7.5),163(6.7),205(1.9),145(0.9)	Forsythoside D or other unknown isomer
Pg3	16.70	FF	477.1603	477.1602	-0.099	$C_{20}H_{29}O_{13}$	MS^2[477]:315(100),221(9.1),135(8.3),179(7.9),143(3.3),459(3.2),161(2.8)	new compound
Pg4	21.65	FF	299.1125	299.1129	1.072	$C_{14}H_{19}O_7$	MS^2[299]:179(100),119(71.7),113(69.2),143(58.3),161(52.1),101(16.7),131(16.2)	Salidroside
Pg5	22.84	FF	461.1654	461.1651	-0.483	$C_{20}H_{29}O_{12}$	MS^2[461]:315(100),135(59.8),281(56.4), 137(33.0) MS^3[315]:135(100),119(5.0),179(4.3),143(2.5)	Forsythoside E
Pg6	34.81	FF	639.1920	639.1915	-0.769	$C_{29}H_{35}O_{16}$	MS^2[639]:621(100),469(7.0),459(1.6),487(1.2) MS^3[621]:469(100),459(93.0),441 (45.2), 179(17.6), 487 (15.7)	(R)-Suspensaside
Pg7	36.70	FF	639.1920	639.1912	-1.160	$C_{29}H_{35}O_{16}$	MS^2[639]:621(100),529(7.3),469(1.8),487(1.6),459(1.0),441(0.7) MS^3[621]:469(100),459 (63.8),441(49.0), 179(17.9)	(S)-Suspensaside
Pg8	39.92	RRP	785.2499	785.2494	-0.611	$C_{35}H_{45}O_{20}$	MS^2[785]:623(100),605(1.1) 459(0.3),639(0.3) MS^3[623]:461(100),477(75.9),459(21.2),315(11.7),297(2.6),44	Echinacoside

							3(1.6),605(1.4),221(0.8)	
Pg9	40.93	FF	639.1920	639.1914	-0.956	$C_{29}H_{35}O_{16}$	MS^2[639.19]:621(100),529(12.8),477(12.6), 487(4.2) MS^3[621]:469(100),459(93.0),441(45.2),487(15.7)	β-hydroxyacteoside
Pg10	43.27	FF	639.1920	639.19196	-0.002	$C_{29}H_{35}O_{16}$	MS^2[639]:477(100),459(11.1),315(2.8),461(1.2),503(1.1) MS^3[477]:315(100),221(11.2),135(8.9),143(5.7),179(5.2),161(3.2)	new compound
Pg11	45.54	FF	609.1814	609.1810	-0.700	$C_{28}H_{33}O_{15}$	MS^2[609]:447(100),429(13.1),315(4.0),477(3.5),179(2.5),473(2.1) MS^3[447]:315(100),135(19.5),149(3.0),191(2.7),131(2.6)	Calceolarioside C or other unknown isomer
Pg12	45.90	RRP	799.2655	799.2648	-0.863	$C_{36}H_{47}O_{20}$	MS^2[799]:623(100),605(10.0),461(1.6),637(1.6),477(0.7),459(0.6),653(0.3),443(0.3) MS^3[623]:461(100),477(74.7),459(20.1),315(11.6),297(2.3),605(1.9),443(1.7)	Jionoside A
Pg13	47.68	FF	623.1970	623.1959	-1.904	$C_{29}H_{35}O_{15}$	MS^2[623]:461(100),443(15.1),487(3.5),477(3.3),178(1.9) MS^3[461]:315(100),135(56.8),205(28.3),163(18.0),145(5.1),143(4.5)	Forsythoside H
Pg14	48.08	FF	609.1814	609.1815	0.203	$C_{28}H_{33}O_{15}$	MS^2[609]:447(100),429(6.38),477(5.61),315(3.21),179(1.44),473(1.05) MS^3[447]:315(100),135(29.59),149(3.53),131(2.79),191(1.99)	Calceolarioside C or other unknown isomer
Pg15	48.18	FF	477.1391	477.1398	1.325	$C_{23}H_{25}O_{11}$	MS^2[477]:161(100),315(17.5),179(11.7),203(4.6),135(1.9),323(1.3),341(1.0)	Calceolarioside A or other unknown isomer
Pg16	49.39	FF	609.1814	609.1812	-0.306	$C_{28}H_{33}O_{15}$	MS^2[609]:447(100),429(7.8),315(4.1),477(2.8),179(1.7),473(1.5) MS^3[447]:315(100),135(21.8),149(5.4),191(4.0),131(2.4)	Calceolarioside C or other unknown isomer

Pg17[a]	49.50	FF	755.2393	755.2387	-0.788	$C_{34}H_{43}O_{19}$	MS^2[755]:593(100), 461(1.6),623(1.6),575(1.5) MS^3[593]:447(100),461(87.2),429(21.4),315(11.6),297(2.3),443(1.8)	Forsythoside B
Pg18	50.21	FF	755.2393	755.2389	-0.550	$C_{34}H_{43}O_{19}$	MS^2[755]:593(100), 461(1.6),623(1.6),575(1.5) MS^3[593]:447(100),461(87.2),429(21.4),315(11.6),297(2.3),443(1.8)	Other unknown isomer of forsythoside B
Pg19	51.15	FF	623.1970	623.1953	-2.883	$C_{29}H_{35}O_{15}$	MS^2[623]:461(100),443(8.7),487(2.3),203(1.6),477(1.5),179(1.5),315(1.1),205(0.3) MS^3[461]:315(100),135(55.2),205(37.3),163(16.6),143(7.8),145(5.5),134(3.0),162(1.3)	Forsythoside I
Pg20	51.74	FF	477.1391	477.1390	-0.268	$C_{23}H_{25}O_{11}$	MS^2[477]:161(100),459(22.7),179(22.1),315(19.2),271(13.2),271(4.4),433(4.4)	Calceolarioside A or other unknown isomer
Pg21[a]	51.78	FF	623.1970	623.1957	-2.097	$C_{29}H_{35}O_{15}$	MS^2[623]:461(100),443(8.7),487(2.3), 477(1.5) MS^3[461]:315(100),135(62.9),205(34.6),163(21.3)	Forsythoside A
Pg22[a]	53.68	FF, RRP, PH	623.1970	623.1963	-1.214	$C_{29}H_{35}O_{15}$	MS^2[623]:461(100),443(3.53), 315(1.7),477(1.6) MS^3[461]:315(100),135(52.9),297(14.9),161 (8.5),143(3.6),163(1.4)	Acteoside
Pg23	54.05	FF	769.2550	769.2538	-1.528	$C_{35}H_{45}O_{19}$	MS^2[769]:607(100),461(2.7),589(2.2),623(1.6),750(1.2),725(1.1),751(1.0),443(0.6)	Forsythoside G
Pg24	55.20	RRP	813.2812	813.2802	-1.180	$C_{37}H_{49}O_{20}$	MS^2[813]:637(100),619(25.6),473(4.4),491(4.3),475(2.3),475(2.2),667(1.4),651(1.3) MS^3[637]:491(100),473(44.1),475(39.6),457(3.0),329(2.6),619(1.9)	Jionoside B

Pg25	54.24	FF	621.1814	621.1808	-0.977	$C_{29}H_{33}O_{15}$	MS^2[621]:469(100),459(65.7),441(51.4),202(30.6),178(21.5),487(18.4),233(14.8),205(6.2),427(5.6),397(3.2) MS^3[469]:179(100),161(89.7),233(68.6),203(31.1),245(19.3),367(14.9),451(14.4),409(7.4),189(8.0)	Suspensaside A or other unknown isomer
Pg26[a]	54.69	FF	477.1391	477.1397	1.073	$C_{23}H_{25}O_{11}$	MS^2[477]:161(100),315(21.5),281(2.7),251(1.9),179(1.8),221(1.1),341(0.7) MS^3[161]:133(100),161(5.9),117(0.6),105(0.4)	Calceolarioside B
Pg27	56.95	FF	621.1814	621.1810	-0.590	$C_{29}H_{33}O_{15}$	MS^2[621]:469(100),459(89.0),487(59.4),203(41.9),441(37.4),427(23.2),179(20.7),397(6.7),367(4.7) MS^3[469]:179(100),161(81.6),233(28.3),135(20.6),367(19.9),203(18.7),451(13.9),409(12.5),263(11.6)	Suspensaside A or other unknown isomer
Pg28[a]	57.83	RRP, PH	623.197	623.1962	-1.407	$C_{29}H_{35}O_{15}$	MS^2[623]:461(100),477(1.5),443(0.8), 179(0.4) MS^3[461]:315(100),135(49.6),297(16.4),169(13.5),143(3.6),134(3.4)	Isoacteoside
Pg29	62.69	PH	637.2127	637.2125	-0.371	$C_{30}H_{37}O_{15}$	MS^2[637]:461(100),491(8.6),443(6.7),475(5.2),593(1.7),329(1.3),315(1.3)	Leucosceptoside A or other unknown isomer
Pg30	67.60	FF	621.1814	621.1810	-0.687	$C_{29}H_{33}O_{15}$	MS^2[621]:459(100),251(44.2),323(34.5),469(32.3),487(27.7),179(22.8),305(18.3) MS^3[459]:151(100),161(41.9),205(20.3),247(15.7),313(13.1),143(9.3),277(8.3),369(7.6),307(7.4)	Suspensaside A or other unknown isomer
Pg31	68.43	PH	637.2127	637.2121	-1.031	$C_{30}H_{37}O_{15}$	MS^2[637]:461(100),491(10.8),475(5.12),538(4.0),265(1.8),315(1.6),443(1.6) MS^3[461]:315(100),135(49.9),161(10.5),297(9.4),143(4.7),307(1.1),179(0.8)	Leucosceptoside A or other unknown isomer

Pg32	74.55	PH	651.2283	651.2279	-0.671	$C_{31}H_{39}O_{15}$	MS[2][651]:475(100),505(35.4),457(22.6),193(18.2),265(9.9),329(7.8),487(5.8)	Cistanoside D or other unknown isomer
Pg33	79.35	PH	651.2283	651.2283	-0.210	$C_{31}H_{39}O_{15}$	MS[2][651]:475(100),505(79.2),193(28.9),265(19.3),487(13.9),457(12.5),337(5.6)	Cistanoside D or other unknown isomer
Pa1	3.06	FLJ	191.0550	191.0556	3.221	$C_7H_{11}O_6$	MS[2][191]:127(100), 173(69.6),93(50.5),171(25.6) MS[3][127]:85(100),109(67.8),99(41.9)	Quinic acid
Pa2[a]	20.44	FLJ	353.0867	353.0861	-1.667	$C_{16}H_{17}O_9$	MS[2][353]:191(100),179(45.4),135(8.5),173(2.7) MS[3][190.97]:127(100),173(73.3),111(35.5),171(30.3)	3-*O*-caffeoylquinic acid
Pa3	26.34	FLJ	337.0918	337.0929	3.281	$C_{16}H_{17}O_8$	MS[2][337]:163(100),191(6.1),119(6.1),173(4.2),293(1.5)	3-*p*-CoQA
Pa4[a]	28.17	FLJ	353.0867	353.0865	-0.534	$C_{16}H_{17}O_9$	MS[2][353]:191(100),179(3.0), 135(0.6),161(0.3) MS[3][191]:127(100),85(77.0), 173(66.4)	5-*O*-caffeoylquinic acid
Pa5[a]	29.75	FLJ	353.0867	353.0865	-1.157	$C_{16}H_{17}O_9$	MS[2][353]:173(100),179(52.0),191(15.0),135(8.2),155(1.5) MS[3][173]:93(100),111(54.4),71(23.0),155(15.7),109(8.1)	4-*O*-caffeoylquinic acid
Pa6	35.68	FLJ	337.0918	337.0929	2.896	$C_{16}H_{17}O_8$	MS[2][337]:191(100),163(6.1), MS[3][191]:127(100),173(71.2),111(35.7),171(29.2)	5-*p*-CoQA
Pa7	36.82	FLJ	337.0918	337.0929	3.163	$C_{16}H_{17}O_8$	MS[2][337]:173(100),163(8.2), 191(5.1), 137(0.9) MS[3][173]:93(100),111(49.4), 155(9.4),137(8.2)	4-*p*-CoQA
Pa8	39.62	FLJ	367.1024	367.1027	0.821	$C_{17}H_{19}O_9$	MS[2][367]:191(100),173(15.0), 193(6.8) MS[3][191]:127(100),173(75.7),93(59.0),171(29.2)	5-feruloylquinic acid

Pa9[a]	58.16	FLJ	515.1184	515.1182	-0.335	$C_{25}H_{23}O_{12}$	MS^2[515]:353(100),335(12.7),179(9.3),191(5.0) MS^3[353]:173(100),179(70.7),191(48.0),135(11.2),155(1.6)	3,4-*O*-dicaffeoylquinic acid
Pa10[a]	60.26	FLJ	515.1184	515.1187	0.597	$C_{25}H_{23}O_{12}$	MS^2[515]:353(100),191(1.8),179(1.5),335(1.1) MS^3[353]:191(100),179(45.7),135(8.4),173(6.3),161(0.8)	3,5-*O*-dicaffeoylquinic acid
Pa11[a]	65.05	FLJ	515.1184	515.1179	-0.937	$C_{25}H_{23}O_{12}$	MS^2[515]:353(100),173(5.8),255(5.4),317(4.4) MS^3[353]:173(100),179(60.4),191(26.6),135(9.1),155(1.5)	4,5-*O*-dicaffeoylquinic acid
Pa12	69.42	FLJ	529.1341	529.1342	0.316	$C_{26}H_{25}O_{12}$	MS^2[529]:367(100),173(20.6),335(17.2),193(3.6),353(3.3),179(2.1)	Methylated dicaffeoylquinic acid
Pa13	76.47	FLJ	529.1341	529.1341	0.203	$C_{26}H_{25}O_{12}$	MS^2[529]:353(100),367(32.5), 203(7.7),173(6.0),191(3.0),179(2.9),349(1.7),193(1.0)	Methylated dicaffeoylquinic acid
F1[a]	50.47	FLJ, FF	609.1450	609.1455	0.819	$C_{27}H_{29}O_{16}$	MS^2[609]:301(100),300(35.0),343(7.7) MS^3[301]:179(100),151(84.7),273(15.2),257(15.2),199(7.7),229(7.0),283(5.5),255(5.1),107(4.9)	Rutin
F2[a]	52.99	FLJ, FF, PH	463.0871	463.0869	-0.415	$C_{21}H_{19}O_{12}$	MS^2[463]:301(100),300(30.3),343(2.6),271(1.6) MS^3[301]:179(100),151(74.4),273(14.8),257(12.5),193(7.0),283(5.9),121(1.8)	Isoquercitrin
F3[a]	53.11	FLJ, FF	447.0922	447.0928	1.436	$C_{21}H_{19}O_{11}$	MS^2[447]:285(100),327(2.0),403(1.1),359(0.7) MS^3[285]:241(100),199(99.5),217(77.8),243(70.9),151(40.4) ,133(16.0),107(9.9)	Luteoloside
F4[a]	53.71	FLJ	593.1501	593.1501	-0.011	$C_{27}H_{29}O_{15}$	MS^2[593]:285(100),284(24.8) MS^3[285]:241(100),199(97.8),175(84.8),243(69.2),217(66.6),151(41.4),,267(25.9),257(23.1),133(8.4)	Lonicerin

F5[a]	57.43	FLJ	593.1501	593.1506	0.798	$C_{27}H_{29}O_{15}$	MS^2[593]:285(100),447(10.2),229(3.4),257(3.3),327(2.4),267(1.8) ,241(0.9)	Kaempferol 3-*O*-rutinoside
F6	61.44	FLJ	477.1028	477.1036	1.797	$C_{22}H_{21}O_{12}$	MS^2[477]:314(100),315(34.5), 271(5.3),273(3.6),300(3.1),299(5.8),151(3.8),179(1.5)	Isorhamnetin-*O*-hexoside
F7[a]	61.51	PH	431.0972	431.0981	1.802	$C_{21}H_{19}O_{10}$	MS^2[431]:269(100), 387(5.1),311(3.1) MS^3[269]:225(100),268(84.4),197(44.7),227(40.5),224(40.3),183 (40.0),149(37.5),169(35.1)	Apigetrin
F8	62.77	FLJ	607.1657	607.1660	0.335	$C_{28}H_{31}O_{15}$	MS^2[607]:299(100),284(39.3),443(5.8),285(4.2),487(3.0)	chrysoeirol-7-*O*-neohesperidoside
F9	63.05	FLJ	637.1763	637.1765	0.218	$C_{29}H_{33}O_{16}$	MS^2[637]:461(100),491(6.3), 443(6.2),329(5.8),475(4.4),193(1.8)	Tricin-7-*O*-neohesperidoside
F10[a]	63.51	FLJ	461.1078	461.1089	2.217	$C_{22}H_{21}O_{11}$	MS^2[461]:299(100),446(73.5),298(12.3),284(10.3) MS^3[299]:284(100),297(0.7),269(0.6),285(0.2),255(0.2),271(0.2),219(0.1),187(0.1),199(0.1)	Diosmetin-7-*O*-glucoside
F11[a]	63.53	FLJ	491.1184	491.1190	1.196	$C_{23}H_{23}O_{12}$	MS^2[491]:476(100),329(57.3),328(11.9),314(11.1) MS^3[476]:343(100),314(95.5),461(53.72),313(34.8),315(24.9), 327(13.0)	Tricin-7-*O*-glucoside
L1	45.78	FF	535.1810	535.1810	0.051	$C_{26}H_{31}O_{12}$	MS^2[535]:373(100),313(3.3),343(2.6),371(0.4),267(0.4),517(0.8) MS^3[373]:313(100),343(55.8),325(7.2),358(3.3),355(0.8),310 (0.4),181(0.1)	(+)-1-hydroxylpinoresinol-*O*-glucoside
L2	55.76	FF	519.1861	519.1860	-0.093	$C_{26}H_{31}O_{11}$	MS^2[519]:357(100),389(0.6),399(0.5) MS^3[357]:151(100),136(38.1),311(13.0),342(10.3),327(3.3),175(2.9)	(+)-Pinoresinol-*O*-glucoside

L3	59.29	FF	519.1861	519.1872	2.122	$C_{26}H_{31}O_{11}$	MS^2[565]:357(100),519(25.1) MS^3[357]:151(100),136(38.1),311(13.0),342(10.3),327(3.3),175(2.9)	(+)-Epipinoresinol -4"-*O*-glucoside
L4	60.64	FF	519.1861	519.1874	2.488	$C_{26}H_{31}O_{11}$	MS^2[565]:357(100),519(35.5), 521(2.2),547(1.8) MS^3[357]:151(100),136(38.1),311(13.0),342(10.3),327(3.3),175(2.9)	(+)-Epipinoresinol -4'-*O*-glucoside
L5	63.97	FF	373.1282	373.1285	0.725	$C_{20}H_{21}O_7$	MS^2[373]:313(100),343(55.2),325(4.7),358(2.9),355(0.4),327(0.3) MS^3[313]:298(100),136(6.2),188(4.6),189(3.5),108(2.1),174(1.2)	(+)-1-hydroxylpinoresinol
L6	65.85	FF	519.1861	519.1864	0.601	$C_{26}H_{31}O_{11}$	MS^2[519]:357(100),353(0.3),399(0.3),501(0.1),355(0.1),313(0.1) MS^3[357]:313(100),342(52.3),209(45.4),298(42.2),147(32.3),281(17.0),162(7.5)	Matairesinoside
L7	70.18	FF	533.2017	533.2032	2.761	$C_{27}H_{33}O_{11}$	MS^2[579]:371(100),263(99.5),296(29.2),533(20.7),248(18.5),233(17.2)	pinoresinol monomethyl ether *O*-glucoside
L8	74.27	FF	371.1489	371.1488	-0.390	$C_{21}H_{23}O_6$	MS^2[371]:356(100),326(3.1),341(1.0),327(0.9),151(0.5) MS^3[356]:121(100),135(57.0),136(30.7),177(30.1),122(26.4),341(22.6),163(20.6),151(14.4)	Phillygenin
L9[a]	74.27	FF	533.2017	533.2030	2.385	$C_{27}H_{33}O_{11}$	MS^2[579]:371(100),533(29.5),543(3.3),207(1.5) MS^3[371]:356(100),326(2.0),341(1.1),323(0.6)	Phillyrin
I1	13.35	RRP	523.1657	523.1656	-0.299	$C_{21}H_{31}O_{15}$	MS^2[523]:179(100),361(36.8),181(35.4),343(27.3) 487(6.4),199(5.9)	Rehmannioside A or B
I2	18.71	FLJ, FF, RRP	375.1285	375.1286	0.151	$C_{16}H_{23}O_{10}$	MS^2[375]:213(100),151(6.1),315(4.2),285(2.8) MS^3[213]:124(100),169 (20.9),151(8.7)	loganin acid

I3	22.67	FLJ	373.1129	373.1127	-0.518	$C_{16}H_{21}O_{10}$	MS^2[373]:211(100),167(41.7),149(15.6),193(11.5),123(9.9)	secologanic acid or other unknown isomer
I4	23.70	FLJ	375.1285	375.1287	0.471	$C_{16}H_{23}O_{10}$	MS^2[375]:213(100),169(15.4),151(3.6), 125(1.7) MS^3[213]:169(100),125(19.6),151(12.6),107(10.9)	8-epi-loganin acid
I5	28.28	FLJ	389.1078	389.1078	-0.123	$C_{16}H_{21}O_{11}$	MS^2[389]:345(100),209(29.0),121(19.5),165(13.8) MS^3[345]:165(100),183(76.7),179(47.3),113(42.4),119(39.3)	secologanoside
I6	29.18	FLJ	373.1129	373.1131	0.474	$C_{16}H_{21}O_{10}$	MS^2[373]:193(100),149(30.8),167(5.4),179(2.8),123(1.7) MS^3[193]:149(100),93(7.2),121(2.5),131(2.1),107(1.0)	secologanic acid or other unknown
I7[a]	34.16	FLJ	357.1180	357.1190	2.776	$C_{16}H_{21}O_9$	MS^2[403]:357(100),195(55.4),179(45.8),125(17.7) MS^3[357]:125 (100),195(46.1),151(12.3),167(9.7)	Sweroside
I8[a]	40.33	FLJ	403.1235	403.1239	1.097	$C_{17}H_{23}O_{11}$	MS^2[403]:371(100),179(21.5),121 (3. 7),191(2.4) MS^3[371]:121(100),165(71.0),209(20.7),181(16.8),311(7.6)	Secoxyloganin
I9	43.94	FLJ	417.1391	417.1397	1.299	$C_{18}H_{25}O_{11}$	MS^2[417]:341(100),237(14.8), 179(8.5),385 (5.5)	Dimethyl-secologanoside
S1[a]	80.89	FLJ	1235.6055	1235.6047	-0.634	$C_{59}H_{95}O_{27}$	MS^2[1236]:1192(90.4),1074(78.5),912(23.5),928(22.1),735(19.7),1056(27.1),977(21.1),1173(16.4)	Macranthoidin A

Table 2

Identification of chemical constituents of XEQJ by LTQ-Orbitrap (Positive Ion Mode)

No	t_R(min)	Component Herb	Theoretical Mass *m/z*	Experimental Mass *m/z*	Error(ppm)	Formula $[M+H]^+$/ $[M+Na]^+$	(+)-ESI-MS^n data,P-ion(%)	Identification
A1	18.55	CL	531.3177	531.3156	-4.068	$C_{28}H_{43}N_4O_6$	MS^2[531]:293(100),222(40.9),165(3.5),367(1.6) MS^3[293]:222(100),165(1)	Kukoamine B
A2	49.49	CL	302.1389	302.1379	-2.564	$C_{17}H_{20}NO_4$	MS^2[302]:121(100),138(98.5),123(1.7),165(1.3) MS^3[121]:93(100),103(11.9),91(10.9),121(1.5)	Dihydro-*N*-caffeo yltyramine
A3	60. 33	CL	634.2759	634.2743	-2.584	$C_{34}H_{40}N_3O_9$	MS^2[634]:504(100),497(33.1),339(12.1),394(11.6),476(7.1) MS^3[504]:394(100),476(77.6),339(51.3),231(50.4)	(1,2-trans)-*N*^{3}-(4-acetamidobutyl)-1-(3,4-dihydroxyp henyl)-7-hydroxy-*N*^{2}-(4-hydroxyphe nethyl)-6,8-dimethoxy-1,2 -dihydro-naphthal ene-2,3-dicarboxa mide
A4	67.25	CL	874.373	874.3707	-2.658	$C_{42}H_{52}N_9O_{12}$	MS^2[874]:856(100),503(10.0),486(6.1) MS^3[856]:468(100),424(11.1),422(10.2),	Lyciumin A
A5	74.29	CL	314.1387	314.1380	-2.179	$C_{18}H_{20}NO_4$	MS^2[314]:177(100),145(9.4),117(0.9) MS^3[177]:145(100),117(0.7),149(0.6)	Trans-*N*-feruloylt yramine
A6	80.89	CL	897.389	897.3862	-3.063	$C_{44}H_{53}N_{10}O_{11}$	MS^2[897]:879(85.2),503(9.8) MS^3[879.40]:468.23(100),671.30(99.8),424(24.6),852(21.7),42 2(10.6)	Lyciumin B
A7	80.94	CL	641.2494	641.2473	-3.286	$C_{36}H_{37}N_2O_9$	MS^2[641]:504(100),476(48.4),339(13.2),394(11.6),231(4.4)	7-hydroxy-1-(3,4-

							MS^3[504]:394(100),476(90.0),231(55.9),339(53.5)	dihydroxy)-N^2,N^3-bis(4-hydroxyphenethyl)-6,8-dimethoxy-1,2-dihydronaphthalene-2,3-dicarboxamide
A8	81.01	CL	643.265	643.2634	-2.452	$C_{36}H_{39}N_2O_9$	MS^2[643]:506(100),343(43.1),505(32.5),325(16.7),625(15.1),293(13.0) MS^3[506]:293(100),325(79.0),247(17.6),369(14.2),310(13.0),341(12.1)	(E)-2-(4,5-dihydroxy-2-{3-[(4-hydroxyphenethyl)amino]-3-oxopropyl}phenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)-*N*-(4-hydroxyphenethyl)acrylamide
A9	81.23	CL	964.4199	964.4180	-1.985	$C_{49}H_{58}N_9O_{12}$	MS^2[964]:946(100),576(10.0),756(7.1),558(4.6) MS^3[946]:558(100), 514(18.1),512(9.6)	Lyciumin C
A10[a]	84.15	IN	263.0815	263.0811	-1.726	$C_{16}H_{11}N_2O_2$	MS^2[263]:219(100),235(30.9),245(24.8),217(9.0) MS^3[219]:219(100),201(3.5),192(3.4),190(2.7)	Indirubin
S2	83.10	CARR	543.2565	543.2551	-2.547	$C_{28}H_{40}O_9Na$	MS^2[543]:497(100),525(12.7),515(7.2),524(6.1) MS^3[497]:479(100),480(40.9),452(37.3),302(30.7)	Glaucogenin C-*O*-*β*-D-thevetopyranoside
S3	83.71	CARR	673.3195	673.3178	-2.443	$C_{34}H_{50}O_{12}Na$	MS^2[673]:627(100),543(7.5),655(5.2), MS^3[627]:497(100),297(57.6),315(27.2),353(1.7)	Cynaversicoside F
S4	84.21	CARR	831.4137	831.4125	-1.518	$C_{42}H_{64}O_{15}Na$	MS^2[831]:785(100),687(5.1),641(3.1),311(2.6)	Cynaversicoside

							MS^3[785]:641(100),311(51.7),497(19.1)	A
S5	84.40	CARR	687.3351	687.3328	-3.402	$C_{35}H_{52}O_{12}Na$	MS^2[687]:641(100),543(53.2),669(17.2),497(14.2)	Atratoglaucoside
							MS^3[641]:497(100),311(42.0),329(14.2),497(5.3)	A
S6	84.88	CARR	817.3981	817.3957	-2.939	$C_{41}H_{62}O_{15}Na$	MS^2[817]:771(100),673.(74.0),543.35(3.6)	Glaucoside C
							MS^3[771]:627(100),441(8.3),297(5.6),497(4.1),353(0.3)	