RESEARCH ARTICLE

Chemical Profiling of Re-Du-Ning Injection by Ultra-Performance Liquid Chromatography Coupled with Electrospray Ionization Tandem Quadrupole Time-of-Flight Mass Spectrometry through the Screening of Diagnostic Ions in MS<sup>E</sup> Mode

Haibo Li<sup>1,2,3,✉</sup>, Yang Yu<sup>2,✉</sup>, Zhenzhong Wang<sup>1,3</sup>, Jianliang Geng<sup>1</sup>, Yi Dai<sup>2</sup>, Wei Xiao<sup>1,3,*</sup>, Xinsheng Yao<sup>2,4,✉</sup>

<sup>1</sup>Jiangsu Kanion Pharmaceutical Co. Ltd., Lianyungang, China, 2 Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou, China, 3 State Key Lab of New-Tech for Chinese Medicine Pharmaceutical Process, Lianyungang, China, 4 Shenyang Pharmaceutical University, Shenyang, China

✉ These authors contributed equally to this work.
* tyaoxs@jnu.edu.cn (XY); kanionxw2010@126.com (WX)

Abstract

The broad applications and mechanism explorations of traditional Chinese medicine prescriptions (TCMPs) require a clear understanding of TCMP chemical constituents. In the present study, we describe an efficient and universally applicable analytical approach based on ultra-performance liquid chromatography coupled to electrospray ionization tandem quadrupole time-of-flight mass spectrometry (UPLC-ESI-Q/TOF-MS) with the MS<sup>E</sup> (E denotes collision energy) data acquisition mode, which allowed the rapid separation and reliable determination of TCMP chemical constituents. By monitoring diagnostic ions in the high energy function of MS<sup>E</sup>, target peaks of analogous compounds in TCMPs could be rapidly screened and identified. “Re-Du-Ning” injection (RDN), a eutherapeutic traditional Chinese medicine injection (TCMI) that has been widely used to reduce fever caused by viral infections in clinical practice, was studied as an example. In total, 90 compounds, including five new iridoids and one new sesquiterpene, were identified or tentatively characterized by accurate mass measurements within 5 ppm error. This analysis was accompanied by MS fragmentation and reference standard comparison analyses. Furthermore, the herbal sources of these compounds were unambiguously confirmed by comparing the extracted ion chromatograms (EICs) of RDN and ingredient herbal extracts. Our work provides a certain foundation for further studies of RDN. Moreover, the analytical approach developed herein has proven to be generally applicable for profiling the chemical constituents in TCMPs and other complicated mixtures.

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Introduction

Traditional Chinese medicine prescriptions (TCMPs), which are combinations of several medicinal herbs, have been widely employed for thousands of years in China and other Asian countries. In clinical practice, TCMPs often exhibit significant advantages of low therapeutic risk and remarkable effect for some chronic, multifactorial, and systemic diseases [1–4]. However, due to the extreme complexities of multiple TCMP components, revealing their pharmacological material basis and mechanism of action remains challenging. Consequently, an effective and reliable analytical approach for the rapid screening and identification of the multiple components contained in TCMPs is in high demand.

Currently, due to its significant advantages in analytical speed and detection sensitivity, ultra-performance liquid chromatography coupled with electrospray ionization tandem quadrupole time-of-flight mass spectrometry (UPLC-ESI-Q/TOF-MS) has become an irreplaceable technique for the online structural elucidation of multiple components in mixtures, especially for complex TCMs/TCMPs, biological samples, and pesticide residues [5–9]. UPLC coupled with MS$^{E}$ ($^{E}$ represents collision energy) technology provides an automated strategy to decrease analysis time and maximize duty cycles by using parallel alternating scans at low collision energy in the collision cell to obtain precursor ion information or at high collision energy to obtain accurate full-scan mass fragment, precursor ion, and neutral loss information. Therefore, both precursor and fragmentation data in exact mass mode were collected in a single run; this method has provided excellent chromatographic and MS efficiencies for the rapid structural elucidation of multiple constituents in complex mixtures [10–12]. In the present study, based on the point of view that a certain type of chemical compounds could produce identical or similar characteristic fragment ions under a suitable collision energy in their tandem mass spectra, a well-designed analytical approach that enabled rapid screening and characterization of multiple TCMP constituents was developed. By virtue of UPLC-ESI-Q/TOF-MS and optimized MS$^{E}$ method, diagnostic fragment ions can be used as invaluable evidence for the detection of both expected and unexpected chemical constituents within TCMPs.

Re-Du-Ning injection (RDN), a traditional Chinese medicine injection (TCMI), was manufactured by Jiangsu Kanion Pharmaceutical Co. Ltd. (Lianyungang, China) and consists of three common herbs: *Lonicera japonica* Thunb. (*L. japonica* Thunb.; Jin-yin-hua), *Gardenia jasminoides* Ellis (*G. jasminoides* Ellis; Zhi-zi) and *Artemisia annua* L. (*A. annua* L.; Qing-hao). In China, RDN is widely used for the treatment of viral infection, such as hand-foot-mouth disease [13–14], influenza [15] and herpes angina efficacy [16]. Although RDN has proven to be clinically effective, the knowledge of its chemical constituents is still limited. The elucidation of the various components contained in RDN is urgently necessary and of great importance to RDN quality control and to understanding its mechanism of action.

In this paper, a robust Waters UPLC-ESI-Q/TOF-MS system and optimized MS$^{E}$ method was utilized, employing RDN as an example for illustration. To our knowledge, this work is the first study on the chemical components contained in RDN using the methodology developed herein. As a result, a total of 90 compounds, including 45 iridoids, 21 organic acids, nine flavonoids, seven lignans, four sesquiterpenes, three coumarins, and one monoterpene were identified or tentatively characterized in RDN. In addition, the source plants of these compounds were confirmed by comparing the extracted ion chromatograms (EICs) of RDN to the corresponding ingredient herbs. This work provides a certain foundation for further studies of RDN. More importantly, this novel approach is expected to be widely applied for analyzing other TCMPs and complex mixtures.
Materials and Methods

2.1. Chemicals and materials

_G. jasminoides_ Ellis, _L. japonica_ Thunb. and _A. annua_ L. were purchased from the Ji’an Medical Material Market (Jiangxi, China). All herbal medicines were identified by Professor Zhou Wu (Jiangsu Kanion Pharmaceutical Co. Ltd.). A voucher specimen was deposited in Jiangsu Kanion Pharmaceuticals (Lianyungang, China). The Re-Du-Ning injection (Batch number: 100906) was manufactured and supplied by Jiangsu Kanion Pharmaceutical Co. Ltd. (Lianyungang, China).

All reference standards were isolated from the RDN injection by various column chromatography techniques and were unambiguously identified by nuclear magnetic resonance (NMR) and MS methods in our laboratory.

Liquid chromatography (LC)-MS-grade acetonitrile and water were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). LC-MS-grade formic acid was obtained from Sigma-Aldrich (St. Louis, USA). The water, methanol and ethanol used for sample extraction were all of analytical grade.

2.2. Sample preparation

The RDN samples were directly evaporated with a rotary evaporator and then diluted to 10 mg/mL. Next, 2 mL of these solutions were transferred into separate clean tubes and dried under nitrogen gas at room temperature. The residues were reconstituted in 2 mL of water and then centrifuged at 10000 rpm for 10 min. Solid-phase extraction (SPE) cartridges, (Vac 3cc, 200 mg, Phenomenex strata C18-E, Torrance, CA) were preconditioned with 3 mL of methanol, followed by 3 mL of water before use. The supernatants were loaded onto the SPE cartridges and washed with 2 mL of water. The SPE cartridges were then eluted with 4 mL of methanol, and the eluents were centrifuged at 10000 rpm for 10 min. Supernatant aliquots of 2 μL were injected into the UPLC/Q-TOF-MS system for analyses.

Ingredient herbal medicine samples (_G. jasminoides_, 2 g; _L. japonica_, 2 g; _A. annua_, 2 g) were immersed in 20 mL of deionized water for 1 h. The solutions were then decocted by boiling 3 times (1 h each time). The extracts were diluted to generate 20 mg/mL solutions. All samples were filtered through a 0.22 μm filter membrane before UPLC-MS analyses.

2.3. UPLC-Q/TOF-MS analyses

UPLC analyses were performed using an ACQUITY UPLC system equipped with a binary solvent system, an automatic sample manager and photodiode array (PDA) detector. The chromatographic separation was performed on an Acquity UPLC BEH C18 Column (3.0 mm × 150 mm, 1.7 μm, waters, Ireland) at a temperature of 40°C. The mobile phases consisted of eluent A (0.1% formic acid in water, v/v) and eluent B (0.1% formic acid in acetonitrile, v/v). These eluents were delivered at a flow rate of 0.4 mL/min with a linear gradient program as follows: 2–5% B from 0 to 5.0 min, 5–12% B from 5.0 to 10.0 min, 12–30% B from 10.0 to 15.0 min, 30–55% B from 15.0 to 19.0 min and 55–100% B from 19.0 to 20.0 min. After maintaining 100% B for 3 min, the column was returned to its initial condition.

The UPLC system was coupled to a hybrid quadrupole, orthogonal time-of-flight (Q-TOF) tandem mass spectrometer (SYNAPT G2 HDMS, Waters, Manchester, U.K.) equipped with ESI. The operating parameters were as follows: capillary voltage of 3 kV (ESI+) or -2.5 kV (ESI-), sample cone voltage of 35 V, extraction cone voltage of 4 V, source temperature of 100°C, desolvation temperature of 300°C, cone gas flow of 50 L/h and desolvation gas flow of 800 L/h. In MS² mode, the trap collision energy for the low-energy function was set at 5 eV, while the ramp trap collision energy for the high-energy function was set at 20–50 eV. Argon
was used as the collision gas for collision-induced dissociation (CID) in MS埃 and MS² modes. To ensure mass accuracy and reproducibility, the mass spectrometer was calibrated over a range of 50–1500 Da using a solution of sodium formate. Leucine-enkephalin (m/z 556.2771 in positive ion mode; m/z 554.2615 in negative ion mode) was used as an external reference for the LockSpray and was infused at a constant flow of 5 μL/min. The data were centroided during acquisition.

Results and Discussion

3.1. Optimization of UPLC and mass spectrometry conditions

The MS埃 acquisition mode required well-resolved peaks to ensure that the predominant fragments were collected from a single precursor ion. Obtaining a desirable chromatographic profile with satisfactory separation and peak shapes without excessive peak tailing was necessary. Different UPLC conditions that included both mobile phase systems (methanol-aqueous and acetonitrile-aqueous) were tested. When the mobile phase was acetonitrile-aqueous, the separation resolution was greatly improved compared to methanol-aqueous. Addition of 0.1% formic acid to the mobile phase reduced peak tailing and enhanced the resolution. Thus, an acetonitrile-aqueous solution with 0.1% formic acid was selected as the mobile phase. In addition, four analytical columns, including the Acquity BEH C18 column (3.0 mm × 150 mm, 1.7 μm), Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 μm), Acquity HSS T3 column (2.1 mm × 50 mm, 1.8 μm) and Acquity Shield PR18 column (2.1 mm × 50 mm, 1.7 μm) were compared to achieve better separation performance. Unfortunately, the results of three different 50 mm columns were not satisfactory as shown in (S1 Fig). Then, we found that the column length could influence the separation efficiency significantly. Thus, the Acquity BEH C18 column (3.0 mm × 150 mm, 1.7 μm) was chosen for analysis in current condition. For mass spectrometry, both positive and negative ion modes were tested, and each target compound type was analyzed in a suitable ESI mode.

3.2. Establishment of the supporting database

A systematic investigation of the chemical constituents in RDN was conducted. A self-built database of compounds that were isolated from three medicinal herb ingredients of RDN was established by retrieving on-line databases or Internet search engines, such as Chemical Abstracts Service (CAS) database, Massbank, Web of Science and ChemSpider. The emphasis was placed on analyzing structural characteristics and MS fragmentation behaviors, especially for diagnostic ions (characteristic fragments). As a result, 259 constituents, including iridoids, organic acids, flavonoids, sesquiterpenes, lignans and coumarins were collected. Five items, compound name, molecular formula, accurate mass, diagnostic fragment ions or neutral losses and UV absorption, were recorded.

3.3. Diagnostic ion screening using the optimized MS埃 method

All chemical constituents in herbal medicine can be categorized into different families based on structural types. Thus, a certain family of compounds with identical carbon skeletons could produce similar characteristic fragment ions under CID conditions in mass spectrometry.

Accordingly, the core idea of our approach is to use the diagnostic ions as markers for target compound detection. To simultaneously generate both precursor and fragment ions using the MS埃 method, low- and high-energy scan functions were switched rapidly and continuously for data acquisition. The high-energy scan function that is used to collect information on fragment ions is generally equivalent to a non-selective MS/MS scan. With such a function, specific
diagnostic ions of diverse compounds contained in TCMPs and their precursor ions and neutral losses were simultaneously collected, providing large quantities of valuable information regarding the structural identification of chemical constituents.

In this study, the screening process of caffeoylquinic acids was considered to describe the approach in detail. Based on the aforementioned self-built database, fragment ions at \( m/z \) 191.0556 and \( m/z \) 179.0340, which can be produced from caffeoylquinic acids as common substructures, were selected as diagnostic ions for detecting other analogues. As shown in (Fig 1), the peaks that appeared in the EIC of the high-energy function of the \( \text{MS}^E \) mode were considered as target compounds and further characterized by accurate mass measurements, MS fragmentation analyses and reference standards. Interestingly, an unexpected compound (labeled as 35) that possessed a novel structure with a rare caffeoylquinic ester acylated at the C-10 position of geniposide was similarly screened out. By comparison, only a few peaks could be detected in the EIC mode of the low-energy \( \text{MS}^E \) function. A wide range of ramped CE (20–50 eV in the present study) in the high-energy \( \text{MS}^E \) function will help reveal the MS fragmentation behaviors of different compounds simultaneously. Similarly, other types of analogues were rapidly screened out by our proposed approach, such as iridoids (S2 Fig), flavonoids (S3 Fig) and others.

3.4. Identification of chemical constituents in RDN

A total of 90 compounds, including 45 iridoids, 21 organic acids, nine flavonoids, seven lignans, four sesquiterpenes, three coumarins and one monoterpenoid were identified or tentatively characterized in RDN (Table 1; S4 Fig). The herb sources of these compounds were confirmed by comparing the base peak chromatograms of RDN to a single herbal extract. The main active constituents of RDN (i.e., caffeoylquinic acids and iridoids) were rapidly screened out by UPLC-ESI/Q-TOF mass spectrometry through diagnostic ion screening with \( \text{MS}^E \). The
| No | t_R  | Selected ion | Elemental composition | Measured mass | Calculated mass | Mass error | MS^E or MS^2 fragmentation | Identification | Source |
|----|------|--------------|-----------------------|--------------|----------------|------------|-----------------|----------------|--------|
| 1  | 6.30 | [M+Na]^+     | C_{16}H_{22}O_{11}   | 413.1056     | 413.1060      | -1.0       | 251.0533, 233.0425; deacetylasperulosidic acid | Gj            |
| 2  | 6.64 | [M+Na]^+     | C_{16}H_{22}O_{10}   | 397.1111     | 397.1111      | 0.0        | 235.0584, 217.0475; gardsoside | Gj            |
| 3  | 7.12 | [M+Na]^+     | C_{16}H_{22}O_{11}   | 415.1216     | 415.1216      | 0.0        | 253.0685, 235.0581, 217.0478, 173.0579; shanzhiside | Gj            |
| 4  | 7.28 | [M+Na]^+     | C_{16}H_{22}O_{10}   | 397.1111     | 397.1111      | 0.0        | 235.0582, 217.0477, 173.0578; geniposidic acid | Gj            |
| 5  | 7.53 | [M+Na]^+     | C_{16}H_{22}O_{11}   | 413.1064     | 413.1060      | 1.0        | 251.0537, 233.0423; monotropein | GJ            |
| 6  | 7.73 | [M+Na]^+     | C_{17}H_{24}O_{11}   | 427.1200     | 427.1216      | -3.7       | 265.0687, 247.0583, 215.0324; deacetylasperulosidic acid methyl ester | Gj            |
| 7  | 7.80 | [M+Na]^+     | C_{17}H_{24}O_{11}   | 429.1363     | 429.1373      | -2.3       | 267.0644, 249.0738, 217.0476; shanzhiside methyl ester | Gj            |
| 8  | 7.86 | [M+Na]^+     | C_{16}H_{22}O_{11}   | 413.1073     | 413.1060      | 3.1        | 251.0536, 233.0422; scandoside | Gj            |
| 9  | 8.30 | [M+Na]^+     | C_{17}H_{24}O_{11}   | 427.1218     | 427.1216      | 0.5        | 265.0687, 247.0583, 215.0324; gardenoside | Gj            |
| 10 | 8.68 | [M+Na]^+     | C_{17}H_{24}O_{10}   | 411.1263     | 411.1261      | 1.0        | 249.0738, 231.0634, 199.0370; 8-epi-apodantheroside | Gj            |
| 11 | 9.01 | [M+Na]^+     | C_{16}H_{22}O_{12}   | 429.1025     | 429.1009      | 2.1        | 267.0485, 249.0378, 217.0115; 8-epi-kingside | Lj            |
| 12 | 9.18 | [M+Na]^+     | C_{17}H_{24}O_{11}   | 429.1369     | 429.1373      | -0.9       | 267.0846, 249.0741, 217.0479; morroniside | Lj            |
| 13 | 9.42 | [M+Na]^+     | C_{16}H_{22}O_{11}   | 413.1058     | 413.1060      | -0.5       | 251.0541, 233.0427; secologanoside | Lj            |
| 14*| 9.56 | [M+Na]^+     | C_{23}H_{34}O_{15}   | 573.1782     | 573.1795      | -2.3       | 411.1268, 249.0740, 231.0633, 199.0372; genipin-1-β-D-gentiobioside | Gj            |
| 15 | 9.78 | [M+Na]^+     | C_{16}H_{22}O_{10}   | 397.1115     | 397.1111      | 1.0        | 235.0584, 217.0475, 199.0371, 173.0214, 147.0056; secologanic acid | Lj            |
| 16 | 9.90 | [M+Na]^+     | C_{16}H_{22}O_{12}   | 429.1010     | 429.1009      | 0.1        | 267.0487, 249.0379, 217.0111; kingside | Lj            |
| 17 | 10.48| [M+Na]^+     | C_{16}H_{22}O_{10}   | 413.1429     | 413.1424      | 1.2        | 251.0894, 233.0788; loganin | Lj            |
| 18*| 10.56| [M+Na]^+     | C_{17}H_{24}O_{10}   | 411.1279     | 411.1267      | 2.9        | 249.0741, 209.0829, 199.0372; geniposide | Gj            |
| 19*| 10.57| [M+H]^+      | C_{23}H_{34}O_{14}   | 597.2195     | 597.2183      | 2.7        | 435.1654, 417.1548, 207.0654, 175.0397; jasminigeniposide | B             |
| 20 | 10.81| [M+Na]^+     | C_{16}H_{22}O_{9}    | 381.1156     | 381.1162      | -1.6       | 219.0632, 201.0525, 173.0577; sweroside | Lj            |
| 21 | 11.02| [M+Na]^+     | C_{17}H_{22}O_{10}   | 409.1100     | 409.1111      | -2.7       | 247.0583, 229.0478; methyl1-(β-O-glucopyranosyloxy)-7-(hydroxymethyl)-1,7a-dihydrocyclopenta[c]pyran-4-carboxylate | Gj            |
| 22*| 11.10| [M+Na]^+     | C_{23}H_{34}O_{12}   | 543.1472     | 543.1478      | -1.1       | 397.1114, 235.0585, 217.0482; 6'-O-trans-p-coumarylgeniposidic acid | Gj            |
| 23*| 11.51| [M+Na]^+     | C_{17}H_{24}O_{11}   | 427.1207     | 427.1216      | -2.1       | 265.0674, 247.0590, 215.0326; sec oxyloganin | Lj            |
| 24*| 11.66| [M+Na]^+     | C_{17}H_{24}O_{10}   | 411.1266     | 411.1267      | -0.2       | 249.0741, 231.0642, 199.0374; 7-epi-vogeloside | Lj            |
| 25*| 11.84| [M+Na]^+     | C_{17}H_{24}O_{10}   | 411.1265     | 411.1267      | -0.5       | 249.0739, 231.0644, 199.0378; vogeloside | Lj            |
| 26*| 11.93| [M+H]^+      | C_{25}H_{32}NO_{11}  | 524.2131     | 524.2132      | -0.2       | 362.1603; L-phenylalanino secologalin | Lj            |
| 27*| 12.04| [M+Na]^+     | C_{17}H_{24}O_{10}   | 411.1269     | 411.1267      | 0.5        | 249.0745, 231.0642, 199.0377; secologanin | Lj            |

(Continued)
Table 1. (Continued)

| No | tR  | Selected ion | Elemental composition | Measured mass | Calculated mass | Mass error | MS² or MS³ fragmentation | Identification | Source* |
|----|-----|--------------|-----------------------|--------------|----------------|------------|--------------------------|----------------|---------|
| 28 | 12.35 | [M+Na]⁺ | C₁₀H₂₀O₁₁ | 453.1388 | 453.1373 | 3.3 | 411.1266, 249.0741, 217.0478; | 6'-O-acetylgeniposide | Gj |
| 29 | 13.03 | [M+H]⁺ | C₁₀H₂₀NO₁₁ | 458.1682 | 458.1662 | 4.4 | 296.1133, 278.1029; | lonijaposide J | Lj |
| 30 | 13.11 | [M+Na]⁺ | C₁₀H₂₀O₁₁ | 441.1375 | 441.1373 | 0.5 | 279.0844, 261.0744, 229.0472; | dimethyl secologanoside | Lj |
| 31 | 13.21 | [M+Na]⁺ | C₂₂H₃₂O₁₄ | 603.1688 | 603.1690 | -0.3 | 422.1191, 260.0663, 242.0554; | 6'-O-trans-sinapoyl gardoside | Gj |
| 32 | 13.72 | [M+H]⁺ | C₁₀H₂₀NSO₈ | 416.1372 | 416.1379 | -1.7 | 254.0852, 236.0746; | xylostosidine | Lj |
| 33 | 13.80 | [M+Na]⁺ | C₁₀H₂₀O₁₁ | 511.1425 | 511.1428 | -0.4 | 411.1254, 249.0742, 231.0712; | 10'-O-succinoylgeniposide | Gj |
| 34 | 13.83 | [M+H]⁺ | C₁₀H₂₀NO₁₀ | 460.1281 | 460.1277 | 0.9 | 298.0751, 280.0646; | jasmigeniposide A | Gj |
| 35* | 14.11 | [M+Na]⁺ | C₃₃H₄₀O₁₈ | 740.2266 | 740.2269 | -0.4 | 541.1472, 523.1427, 209.0824; | (Z)-aldosecologanin | Lj |
| 36 | 14.22 | [M+Na]⁺ | C₁₀H₂₀O₁₁ | 457.1685 | 457.1686 | -0.1 | 295.1157, 277.1054, 263.0895; | secologanin dimethyl acetal | Lj |
| 37* | 14.26 | [M+Na]⁺ | C₃₄H₄₆O₁₉ | 781.2532 | 781.2531 | 0.1 | 619.2036, 549.1576, 517.1330, 387.1042, 355.0804; | (Z)-aldosecologanin | Lj |
| 38 | 14.35 | [M+H]⁺ | C₁₀H₂₀O₁₂ | 521.1658 | 521.1659 | -0.1 | 375.1294, 213.0764, 195.0659; | 2'-O-p-hydroxybenzoyl gardoside | Gj |
| 39 | 14.58 | [M+Na]⁺ | C₃₂H₄₀O₁₆ | 617.1845 | 617.1846 | -0.2 | 411.1267, 249.0801, 231.0639, 199.0382; | 6'-O-trans-p-coumaroylgenipin gentiobioside | Gj |
| 40* | 14.75 | [M+Na]⁺ | C₃₂H₄₀O₁₆ | 779.2378 | 779.2374 | 0.5 | 571.1626, 553.1534, 209.0830; | 6'-O-trans-sinapoylgenipin gentiobioside | Gj |
| 41* | 14.84 | [M+Na]⁺ | C₃₃H₄₂O₁₈ | 781.2532 | 781.2531 | 0.3 | 619.2133, 549.1332, 517.1320, 387.1041, 355.0841; | (E)-aldosecologanin | Lj |
| 42* | 14.97 | [M+Na]⁺ | C₃₃H₄₂O₁₈ | 749.2266 | 749.2269 | -0.4 | 541.1472, 523.1427, 209.0824; | 6'-O-trans-p-feruloylgenipin gentiobioside | Gj |
| 43 | 16.33 | [M+Na]⁺ | C₂₈H₃₄O₁₄ | 617.1845 | 617.1846 | -0.2 | 411.1267, 249.0801, 231.0639, 199.0382; | 6'-O-trans-sinapoylgeniposide | Gj |
| 44* | 18.04 | [M+H]⁺ | C₂₈H₃₄NO₁₀ | 506.2032 | 506.2026 | 0.8 | 344.1493, 326.1419, 298.1437, 274.1083, 256.1046, 228.0637; | L-phenylalanino secologanin B | Lj |
| 45* | 18.45 | [M+Na]⁺ | C₂₈H₃₄O₁₆ | 703.2203 | 703.2214 | -1.6 | 495.1643, 477.1374, 209.0827; | 6'-O-trans-cinnamoylgenipin gentiobioside | Gj |
| 46 | 1.77 | [M+H]⁻ | C₇H₁₂O₆ | 191.0561 | 191.0556 | 2.6 | 173.0466, 137.0236, 129.0551; | quinic acid | Lj/Gj |
| 47* | 7.95 | [M+H]⁺ | C₁₆H₁₈O₉ | 353.0876 | 353.0873 | 0.8 | 191.0559, 179.0439, 129.0553, 161.0235, 135.0452; | 5-O-cafeoylquinic acid | Lj/Gj/Aa |
| 48 | 9.00 | [M+H]⁺ | C₇H₆O₃ | 137.0238 | 137.0239 | -0.7 | Overlapped in MS⁵ chromatogram | salicylic acid | Lj/Gj/Aa |
| 49* | 9.23 | [M+H]⁺ | C₁₆H₁₈O₉ | 353.0879 | 353.0873 | 1.7 | 191.0560, 179.0357, 173.0469, 161.0265, 135.0458; | 3-O-cafeoylquinic acid | Lj/Gj/Aa |
| 50* | 9.54 | [M+H]⁺ | C₁₆H₁₈O₉ | 353.0875 | 353.0873 | 0.6 | 191.0561, 179.0352, 173.0454, 161.0217, 135.0450; | 4-O-cafeoylquinic acid | Lj/Gj/Aa |
| No | \(m_\text{r} \) | Selected ion | Elemental composition | Measured mass | Calculated mass | Mass error | MS\(^E\) or MS\(^S\) fragmentation | Identification | Source
|---|---|---|---|---|---|---|---|---|---|
| 51* | 9.72 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{9}\) | 367.1036 | 367.1029 | 1.9 | 353.0876, 191.0549, 179.0380, 173.0411, 161.0265, 135.0424; | 5-O-cafeoylquinic methyl ester | Lj/Gj/Aa |
| 52* | 10.12 | [M-H]\(^-\) | C\(_{3}\)H\(_{8}\)O\(_{4}\) | 179.0345 | 179.0344 | 0.3 | 135.0445; | trans-cafeic acid | Lj/Gj |
| 53* | 10.33 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{9}\) | 367.1046 | 367.1029 | 4.6 | 353.0786, 191.0527, 179.0388, 173.0450, 161.0259, 135.0468; | 3-O-cafeoylquinic methyl ester | Lj/Gj/Aa |
| 54 | 10.81 | [M-H]\(^-\) | C\(_{16}\)H\(_{16}\)O\(_{8}\) | 367.1042 | 367.1029 | 3.5 | 353.0871, 191.0565, 179.0366, 173.0456, 161.0313, 135.0445; | 3,4-di-O-caffeoylquinic methyl ester | Lj/Gj |
| 55* | 11.26 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{9}\) | 367.1042 | 367.1029 | 3.5 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-caffeoylquinic methyl ester | Lj/Gj |
| 56 | 11.47 | [M-H]\(^-\) | C\(_{9}\)H\(_{8}\)O\(_{4}\) | 179.0345 | 179.0344 | 0.3 | 135.0445; | trans-p-hydroxycinnamic acid | Lj/Gj |
| 57 | 11.94 | [M-H]\(^-\) | C\(_{17}\)H\(_{16}\)O\(_{9}\) | 367.1042 | 367.1029 | 3.5 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-caffeoylquinic methyl ester | Lj/Gj |
| 58* | 12.21 | [M-H]\(^-\) | C\(_{9}\)H\(_{8}\)O\(_{2}\) | 179.0345 | 179.0344 | 0.3 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-caffeoylquinic methyl ester | Lj/Gj |
| 59 | 13.25 | [M-H]\(^-\) | C\(_{9}\)H\(_{10}\)O\(_{4}\) | 181.0500 | 181.0501 | -0.3 | Overlapped in MS\(^E\) chromatogram | syringaldehyde | Gj/Aa |
| 60* | 13.33 | [M-H]\(^-\) | C\(_{25}\)H\(_{24}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | 353.0887, 191.0567, 179.0357, 173.0456, 161.0255, 135.0455; | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 61* | 13.68 | [M-H]\(^-\) | C\(_{25}\)H\(_{24}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 62* | 14.35 | [M-H]\(^-\) | C\(_{25}\)H\(_{24}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 63* | 15.62 | [M-H]\(^-\) | C\(_{25}\)H\(_{24}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 64* | 15.67 | [M-H]\(^-\) | C\(_{25}\)H\(_{24}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 65 | 15.67 | [M-H]\(^-\) | C\(_{17}\)H\(_{16}\)O\(_{8}\) | 367.1042 | 367.1029 | 2.1 | Overlapped in MS\(^E\) chromatogram | trans-ferulic acid | Lj/Gj/Aa |
| 66* | 15.80 | [M-H]\(^-\) | C\(_{17}\)H\(_{16}\)O\(_{8}\) | 367.1042 | 367.1029 | 2.1 | Overlapped in MS\(^E\) chromatogram | trans-ferulic acid | Lj/Gj/Aa |
| 67 | 12.10 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 68 | 12.53 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 69 | 12.67 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 70* | 12.77 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 71 | 13.59 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 72* | 17.53 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 73* | 17.63 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 74* | 17.80 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |
| 75 | 20.00 | [M-H]\(^-\) | C\(_{17}\)H\(_{20}\)O\(_{12}\) | 515.1201 | 515.1190 | 2.1 | Overlapped in MS\(^E\) chromatogram | 3,4-di-O-cafeoylquinic acid | Lj/Gj |

(Continued)
remaining compounds were identified according to their accurate mass measurements within 5 ppm error, tandem MS behaviors, database-matching and reference standards. Both negative and positive ion modes were examined, and the base peak intensity (BPI) profiles of RDN and three ingredient herbs are shown in (Fig 2, S5 and S6 Figs). Recently, ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS) has been widely used to characterize chemical profiling of herbal medicines and TCMPs. This method has become one of the most frequently applied approaches in the area of fast chromatographic separations [17-19]. High-resolution tandem mass spectrometry can provide a more specific and accurate mass as long as the co-eluting compounds possess different m/z values [20]. And the isotopic abundances and the elemental composition of fragment ions are greatly conducive to the structural elucidation of unknown compounds. However, it should be pointed out that identification of chemical components from complex compounds were identified according to their accurate mass measurements within 5 ppm error, tandem MS behaviors, database-matching and reference standards. Both negative and positive ion modes were examined, and the base peak intensity (BPI) profiles of RDN and three ingredient herbs are shown in (Fig 2, S5 and S6 Figs). Recently, ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS) has been widely used to characterize chemical profiling of herbal medicines and TCMPs. This method has become one of the most frequently applied approaches in the area of fast chromatographic separations [17-19]. High-resolution tandem mass spectrometry can provide a more specific and accurate mass as long as the co-eluting compounds possess different m/z values [20]. 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And the isotopic abundances and the elemental composition of fragment ions are greatly conducive to the structural elucidation of unknown compounds. However, it should be pointed out that identification of chemical components from complex
TCMPs relying solely on mass spectrometry-based approaches was insufficient. As the spectral differences for some isomers are very small and they cannot be differentiated and unambiguously identified. Therefore, in present study, some reference standards isolated from the entitled injection were used to validate the elucidation of those isomers. Thus, it provides the enhanced accuracy and reliability of MS quantitative results.

3.4.1. Iridoids

Iridoids are the main constituents of RDN. This category of compounds was primarily derived from *L. japonica* and *G. jasminoides*. In this study, 45 iridoids were identified in positive ion mode. Four of the iridoids were new compounds, which were previously isolated and identified using NMR [21]. The diagnostic fragment ions of these compounds were previously reported [22–24]. Such fragments include the neutral cleavage of the glycosidic bond with the neutral loss of a glucose unit (162 Da) and subsequent losses of H₂O, CO and CH₃OH. As shown in (S2 Fig), 32 peaks appearing in EIC mode were considered as target compounds by extracting the diagnostic ions 209.0814, 251.0532, 235.0582 and 215.0320 with the high-energy MSE function. The proposed fragmentation pathways of typical compounds are discussed in detail below.

Compounds 39, 40, 42 and 45 were unambiguously identified as 6''-O-trans-p-coumaroylgenipin gentiobioside, 6''-O-trans-sinapoylgenipin gentiobioside, 6''-O-trans-feruloylgenipin gentiobioside and 6''-O-trans-cinnamoylgenipin gentiobioside, respectively, by comparing their retention times with authentic reference substances isolated from RDN and fragmentation pathways observed in the MS/MS experiments. Of these, compound 42 was new. Interestingly, we discovered that the MS/MS spectra of their [M+Na]⁺ adducts showed base peaks at m/z 511.1414 (C₂₁H₂₈O₁₂Na), 571.1626 (C₂₃H₃₂O₁₅Na), 541.1472 (C₂₂H₃₀O₁₄Na) and 495.1643 (C₂₁H₂₈O₁₂Na), respectively. All of these peaks were produced by the loss of a C₉H₁₀O₄ fragment (Fig 3). This fragmentation pathway is different from that of iridoid glycosides in which the C₆-C₃ unit is not substituted onto the C-6 position of the glucose unit. We
presumed that the C6-C3 unit, an electron-donating group, might have led to this phenomenon. This mechanism should be further investigated.

Compound 44 showed a [M+H]^+ ion at m/z 506.2032 with an elemental composition of C25H32NO10. The MS/MS spectrum of [M+H]^+ exhibited an obvious fragment ion, [M+H-Glc]^+, at m/z 344.1493 (C19H22NO5) from the loss of a neutral glucose residue (162 Da). The base peak at m/z 274.1083 (C15H16NO4) was formed by a retro-Diels-Alder (RDA) cleavage reaction in the aglycone moiety. This precursor ion (C15H16NO4) further produced two characteristic fragment ions at m/z 256.1046 (C15H14NO3) and 228.1037 (C14H14NO2) through the loss of one H2O and the further loss of one CO, respectively. Moreover, two characteristic fragment ions at m/z 326.1419 (C19H20NO4) and 298.1437 (C18H20NO3) were formed from [M+H-Glc]^+ by the successive losses of H2O and CO. Thus, compound 44 could be tentatively
identified as L-phenylalaninosecologanin B (Fig 4), which was further confirmed by comparison to a reference standard.

Compounds 37 and 41 gave the same molecular formula of C_{34}H_{46}O_{19} from their precursor [M+Na]^{+} ions at m/z 781.2515 and 781.2533, respectively. In (Fig 5a and 5b) illustrated the positive ion mode MS/MS spectra of compounds 37 and 41 at 35 eV and 42 eV trap collision energy, respectively. Their diagnostic fragmentation ions demonstrated minor differences with the exception for their peak intensity. For example, in compound 37, a predominant [M+Na]^{+} ion was observed at m/z 781.2515 (C_{34}H_{46}O_{19}Na, 781.1531, 2.0 ppm). An obvious fragment ion [M+Na-Glc]^{+} at m/z 619.2036 was observed by the neutral loss of 162 Da. The additional loss of CH_{3}OH (32 Da) produced [M+Na-Glc-CH_{3}OH]^{+} at m/z 587.1668. The second predominant peak at m/z 549.1576 (C_{24}H_{30}O_{13}Na) was formed through an retro-Diels-Alder (RDA) reaction with the neutral loss of C_{4}H_{6}O. The fragment ion at m/z 517.1330 was formed by successive loss of another CH_{3}OH molecule from the ion at m/z 549.1576. A minor peak at m/z 387.1042 (C_{17}H_{24}O_{10}Na) was formed by cleavage of another C_{17}H_{23}O_{9} fragment. Further loss of CH_{3}OH (32 Da) from 387.1042 (C_{17}H_{23}O_{10}Na) produced m/z 355.0804 (C_{16}H_{19}O_{9}). Thus, compound 37 was tentatively identified as (E)-aldosecologanin. Compounds 37 and 41 were further confirmed to be (E)-aldosecologanin and (Z)-aldosecologanin, respectively, based on comparing their retention times with the isolated compounds and fragmentation pathways observed in our MS/MS experiments.

The [M+Na]^{+} ion of compound 35 was observed at m/z 747.2114, indicating an elemental composition of C_{33}H_{40}O_{18}Na (747.2115, 0.1 ppm). The MS/MS spectrum of [M+Na]^{+} showed a base peak at m/z 377.0778 (C_{16}H_{18}O_{9}Na) produced by cleavage of the C_{17}H_{22}O_{9} fragment. In addition, neutral loss of a glucose unit (162 Da) generated the [M+Na-Glc]^{+} at m/z 585.1559. Successive losses of CH_{3}OH and H_{2}O molecules formed [M+Na-Glc-CH_{3}OH]^{+} at m/z 553.1147 and [M+Na-Glc-CH_{3}OH-H_{2}O]^{+} at m/z 535.1448. Loss of the C_{16}H_{16}O_{8} fragment produced [M+Na-C_{16}H_{16}O_{8}]^{+} at m/z 411.1322 (C_{17}H_{23}O_{10}Na), and successive loss of another H_{2}O molecule from 411.1322 led to the formation of an obvious ion at m/z 393.0970. The
Fig 5. MS/MS spectra of compounds 37 (a) and 41 (b) and proposed fragmentation pathways of compound 37.

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fragment ion at \( m/z \) 231.1102 \((C_{11}H_{12}O_{4}Na)\) was produced by neutral loss of a glucose unit (162 Da). Other characteristic fragment ions were formed, such as \( m/z \) 215.0157 and 199.0407, by successive or simultaneous losses of an O atom and a CH₃OH molecule from \( m/z \) 231.1102 (Fig 6). Thus, compound 35 was identified as jasmigeniposide A, which was a new compound isolated from RDN. This result was further confirmed through reference standard comparison.

In addition to the above compounds, 37 iridoid glycosides (compounds 1–34, 36, 38 and 43) were identified or tentatively characterized from RDN (Table 1) based on their molecular weights and the tandem fragmentation patterns.

### 3.4.2. Organic acids

According to previous research, caffeoylquinic acids as the main bioactive components in RDN were found in *L. japonica* Thunb., *G. jasminoides* Ellis and *A. annua* L. The structures of these typical constituents generally consist of a quinic acid moiety and mono- or dicaffeic acids that are linked to the 3-OH and/or 4-OH and/or 5-OH \[22\]. These compounds exhibit common proposed fragmentation pathways and diagnostic fragmentation ions, such as \( m/z \) 353, 191, 179, 173, 135, etc. The differences in the diagnostic fragmentation ion intensity could be used to identify their structures. As shown in (Fig 1), 15 peaks, including 14 caffeoylquinic acids and one caffeoylquinic substituted new iridoid glycoside, appeared in EIC mode and were considered as target compounds by extracting diagnostic ions 191.0556 and 179.0340 in the high-energy MS² function.
Six peaks were easily located in the chromatogram of RDN by extracting \( m/z \) 353.0873. Similarly, three parent ions at \( m/z \) 515.1190 were located. By comparison with accurate retention times, the first three ions were assigned as monocaffeic acids, while the latter three were identified as dicaffeic acids (Fig 7). According to the literature [25–27], the linkage position of the caffeoyl groups on quinic acid could be determined according to its MS\(^2\) fragmentation behavior. Briefly, when the caffeoyl group was linked to 3-OH or 5-OH, the [quinic acid-H\(^+\)] ion at \( m/z \) 191 was the base peak, and the [caffeic acid-H\(^+\)] ion at \( m/z \) 179 was more significant for 3-O-caffeoylquinic acids. The [quinic acid-H\(_2\)O-H\(^+\)] ion at \( m/z \) 173 was the prominent peak when the caffeoyl group was linked to 4-OH. In our experiment, this fragmentation behavior was also observed in the negative mode MS\(^{E}\) spectra. Thus, compounds 47, 49 and 50 were identified as 5-O-caffeoylquinic acid, 3-O-caffeoylquinic acid and 4-O-caffeoylquinic acid, respectively. Similarly, compounds 51, 53 and 55 were identified as 5-O-caffeoylquinic methyl ester, 3-O-caffeoylquinic methyl ester and 4-O-caffeoylquinic methyl ester, respectively.

Compound 61 had a base peak ion at \( m/z \) 191.0561 and a secondary peak at \( m/z \) 179.0349. As reviewed above, 61 could be identified as a 3-substituted quinic acid. Therefore, peak 18 was identified as 3,5-di-O-caffeoylquinic acid, which was further confirmed by comparison to a reference standard. Compounds 60 and 62 both produced a base peak at \( m/z \) 173, indicating that they were both 4-substituted quinic acids. According to literature [28], the retention time of 3,4-di-O-caffeoylquinic acid is shorter than that of 4,5-di-O-caffeoylquinic acid, and thus, the compounds were identified as 3,4-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid, respectively. These retention times were consistent with those of the separate compounds. In addition, compounds 63, 64 and 66 were identified as 3,4-di-O-caffeoylquinic methyl ester, 3,5-di-O-caffeoylquinic methyl ester and 4,5-di-O-caffeoylquinic methyl ester, respectively.

Compound 46 was identified as quinic acid by comparison with a standard compound. Compounds 54, 57 and 59 were tentatively assigned as 3-hydroxy-4-methoxy styrene acrylic acid, 3-O-caffeoylshikimic acid and syringaldehyde, respectively, by matching their accurate
molecular weights with those in a chemical database. These assignments were further corroborated by comparison with standard substances. Compounds 48, 52, 56, 58 and 66 were identified by comparison with isolated compounds from RDN (as listed in Table 1).

3.4.3. Flavonoids

The MS/MS behaviors of flavonoids and their glycosides have been extensively described [22, 29–31]. Briefly, the primary MS/MS behavior of aglycones was described by the RDA fragmentation pathway. Successive loss of CO from the ketone group, C-fragmentation and loss of radicals, such as \( \text{CH}_3 \) and \( \text{CHO} \), have been described. For flavonoid glycosides, the glycosidic bond is easily cleaved in positive ion mode, and the neutral loss of 162 Da is the characteristic fragment ion of flavonoid O-glycosides. The fragment ion at \([\text{M+H-308}]^+\) corresponds to the loss of a rutinose unit.

As shown in Table 1, a total of nine flavonoids were screened from RDN, four of which were unambiguously identified as rutin (67), hyperoside (68), luteolin-7-O-\( \beta \)-d-glucoside (70), luteolin (73) and quercetin (74) by comparison with standard constituents isolated from RDN. The other four flavonoids were tentatively identified as lonicerin (69), eriodictyol (71), artemetin (72) and eupatin (75) by matching their extract molecular weights with the chemical database and MS/MS fragmentation behavior.

3.4.4. Identification of other compounds

Another 15 obvious peaks in the extracted ion chromatogram of RDN were identified (Table 1). Three coumarins, compounds 87, 88 and 89, were unambiguously identified as scopoletin, 7-hydroxy-6,8-dimethoxyphenyl coumarin and coumarin, respectively, by comparison with the isolated reference standards. Among the four sesquiterpenes, compound 83 was tentatively assigned as 7,8,11-trihydroxyguai-4-en-3-one 8-\( \beta \)-d-glucopyranoside by matching its mass with the chemical database within 5 ppm. Compounds 84, 85 and 86 were confirmed by matching to the retention times of the isolated reference standards. Similarly, seven lignans (compounds 76, 77, 78, 79, 80, 81 and 82) and one monoterpane (compound 90) were also identified.

Conclusion

In this work, an approach involving UPLC-ESI-Q/TOF-MS coupled with MS\(^E\) data acquisition was developed to profile multiple chemical constituents in RDN. Diagnostic ions were used as invaluable markers for the screening of target compounds. A total of 53 compounds, including two new iridoids, were identified or tentatively characterized using this method. Due to the structural complexity of the chemical constituent types in TCMPs, the present analytical approach still has a limitation in the detection of low-abundance components. The remaining 37 compounds were identified according to their accurate mass measurements within 5 ppm error, tandem MS behaviors, database-matching and reference standards. The RDN herbal sources were unambiguously confirmed by comparing the extracted ion chromatograms (EICs) of RDN and ingredient herbal extracts. The results of our study not only provide a certain foundation for further studies of RDN but also demonstrate chemical profile analyses of TCMPs via UPLC-ESI-Q/TOF-MS and diagnostic ion screening using MS\(^E\).

Supporting Information

S1 Fig. Total ion chromatograms (TIC) of three different types of 50 mm columns. (DOCX)
S2 Fig. MS chromatograms of diagnostic ions: (A) EICs of diagnostic ions 209.0814, 251.0532, 235.0582 and 215.0320 in the high-energy function of MSE; (B) TIC of RDN in the high-energy function of MSE; (C) EICs of diagnostic ions 209.0814, 251.0532, 235.0582 and 215.0320 in the low-energy function of MSE; (D) TIC of RDN in the MSE low-energy function. (DOCX)

S3 Fig. MS chromatograms of diagnostic ions: (A) EICs of diagnostic ions 285.0399 and 301.0348 in the high-energy function of MSE; (B) TIC of RDN in the high-energy function of MSE; (C) EICs of diagnostic ions 285.0399 and 301.0348 in the low-energy function of MSE; (D) TIC of RDN in the MSE low-energy function. (DOCX)

S4 Fig. Structures of identified components in RDN (Red: new compounds) (DOCX)

S5 Fig. Basic peak intensity (BPI) profiles of three individual herbs in positive ion mode. Gj = Gardenia jasminoides Ellis, Lj = Lonicera japonica Thunb. and Aa = Artemisia annua L. (DOCX)

S6 Fig. Basic peak intensity (BPI) profiles of three individual herbs in negative ion mode. Gj = Gardenia jasminoides Ellis, Lj = Lonicera japonica Thunb. and Aa = Artemisia annua L. (DOCX)

S7 Fig. Graphical abstract of our research. (DOCX)

Author Contributions
Conceived and designed the experiments: XSY WX HBL YY. Performed the experiments: HBL YY ZZW JLG. Analyzed the data: HBL YY ZZW JLG. Contributed reagents/materials/analysis tools: YD JLG. Wrote the paper: HBL YY.

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