Analytical Methods

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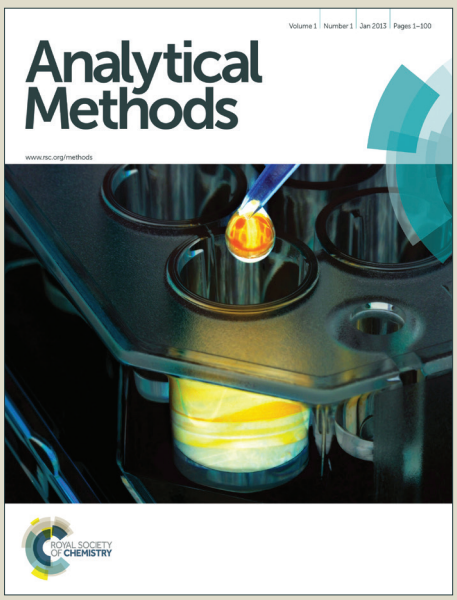

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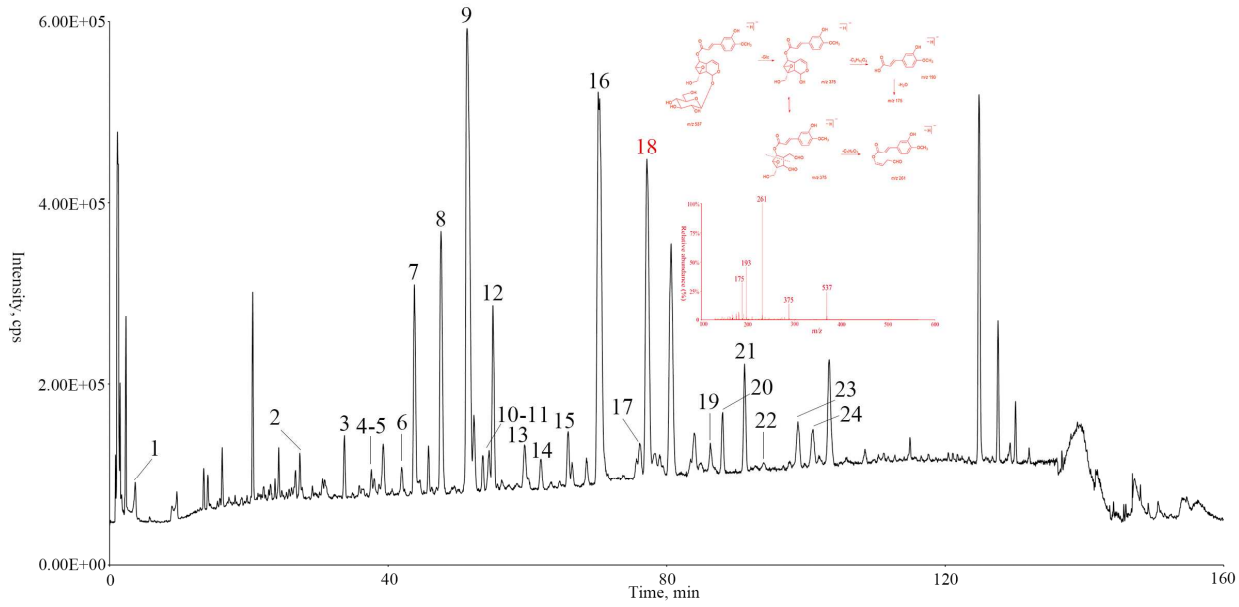
A simple and effective method employing HPLC-QTOF-MS/MS was established for the qualitative analysis of chemical constituents in *Neopicrorhiza scrphulariiflora* roots.



Analytical Methods

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ARTICLE

Characterization and identification of chemical components in *Neopicrorhiza scrphulariiflora* roots by liquid chromatography-electrospray ionization quadrupole time-of-flight tandem mass spectrometry

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High performance liquid chromatography-quadrupole time-of-flight tandem mass spectrometry (HPLC-QTOF-MS/MS) was applied to identify chemical components occurred in *Neopicrorhiza scrphulariiflora* roots. 24 compounds, including 12 iridoid glycosides, 3 phenyl glycosides and 9 phenylethanoid glycosides, were detected and tentatively characterized according to accurate mass measurements and the characteristic fragmentation at low and high collision energy. Meanwhile, appropriate fragmentation pathways were proposed based on definite composition of the product ions. 6′-O-ferulyol-3,4-dihydrocatalpol, 1-[2-(4-(3,4-dihydroxycinnamoyl)-3-glucosyl)glucosy]ethy-3-hydroxy-4-methoxybenzene are potentially novel compounds. 6′-O-caffeoylcatalpol and maxoside were characterized in *N. scrphulariiflora* for the first time. This established HPLC-QTOF-MS/MS method is efficient for identifying and could be the basis for the comprehensive quality control of *N. scrphulariiflora* roots.

Introduction

Neopicrorhiza scrphulariiflora, belonging to Scrophulariaceae family, is distributed in high altitude region (3600-4400 meters) in southeast Tibet and northwest Yunnan in China [1]. Since 1977, it has been officially listed in the Chinese Pharmacopoeia as a substitute for *Picrorhiza kurrooa*. The roots of *N. scrphulariiflora* have been used for the treatment of damp-heat dysentery, jaundice and steaming of bone. Previously phytochemical studies led to the isolation of iridoid glycosides, phenyl glycosides, phenylethanoid glycosides and terpenoids from *N. scrphulariiflora* roots [2-4].

For the analytical investigation of *N. scrphulariiflora* roots, only picroside II has been determined by high pressure liquid chromatography (HPLC)-ultraviolet (UV) detection [5]. Actually, the other components, such as picroside I，picroside IV, scroside A, scrophuloside A and scrophuloside B, were also responsible for the biological activities [6-7]. However, isolation and purification of compounds need time-consumed chromatographic procedures. Structural characterization is also difficult as signals of sugar moieties usually overlap. It is highly desirable to develop a rapid and reliable method for identification of chemical components directly from complex extract of *N. scrphulariiflora* roots without isolation and purification.

High performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) is now a well-established and powerful platform for rapid identification of known compounds as well as elucidation of unknown compounds in crude plant extracts since it could give accurate mass and formulae of non-target compounds. Furthermore, QTOF-MS/MS provides fragmentation analysis and facilitates the elucidation of structures for target and/or non-target compounds. Up to now, a great number of regulations were summarized for characterization and structure elucidation of unknown compounds even without reference standards [8-10].

In present study, a simple method employing High performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) was applied to unequivocally identify chemical components in *N. scrphulariiflora* roots. A total of 24 components were detected, including 12 iridoid glycosides, 3 phenyl glycosides and 9 phenylethanoid glycosides. Characteristic neutral loss and product ions were concluded. Appropriate fragmentation pathways were proposed based on definite composition of the product ions. 6′-O-ferulyol-3,4-dihydrocatalpol and 1-[2-(4-(3,4-dihydroxycinnamoyl)-3-glucosyl)glucosy]ethy-3-hydroxy-4-methoxybenzene are potentially novel compounds. 6′-O-caffeoylcatalpol and maxoside were characterized in *N. scrphulariiflora* for the first time.

Experimental

Reagents

Acetonitrile (Fisher, optima®, LC-MS grade, Fair Lawn, NJ 07410, U.S.A.), formic acid (Merck, EMSURE®, analytical grade, Darmstadt 64271, Germany). Water used in the experiment was deionized and further purified by Milli-Q Plus water purification system (Millipore Ltd., Bedford, MA, USA). Other reagents and chemicals were of analytical grade.

Plant material

Neopicrorhiza scrphulariiflora roots were purchased from Qinghai Jiukang Medicine Corporation Ltd. and identified by Professor Tingnong He (Northwest Institute of Plateau Biology, Chinese Academy of Sciences). Voucher specimens have been deposited at the herbarium of Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

Sample preparation

Dried and finely powdered *N. scrphulariiflora* roots (10 g) were extracted with 70% aqueous acetone (100 mL×3) at room temperature. The extract was concentrated under reduced pressure and then dissolved in 40% aqueous acetonitrile (100 mL). The solution was filtered through a 0.22 μm filter before LC-MS analysis.

HPLC condition

HPLC analysis was performed on an Agilent 1200 series HPLC system (Agilent Technologies, Waldbronn, Germany), coupled to an auto-sampler and a quaternary solvent delivery system with an online degasser.

Chromatographic separation was performed on ACQUITY UPLC® HSS T3 column (100mm × 2.1mm i.d., 1.8 μm, Waters, Massachusetts, U.S.A.). The mobile phase consisted of acetonitrile (A) and 0.1% formic acid/water (B) with a gradient elution of 2-10% A at 0-16min, 10-20% A at 16-96min, 20-34% A at 96-131 min, 34-100% A at 131-135 min and 100% A at 135-160 min. The mobile phase was eluted at a flow rate of 220 μL/min, and injection volume was 2.00 μL.

Mass spectrometry

Mass spectrometry was performed using a QSTAR Elite LC-MS/MS system from Applied Biosystems/MDS Sciex (Concord, ON, Canada) coupled with an electrospray ionization (ESI) interface. Nitrogen was used in all cases. The parameters were optimized as follows: ESI voltage, -4000V; nebulizer gas, 60; auxiliary gas, 50; curtain gas, 35; Turbo gas temperature, 500℃; declustering potential, -60V; focusing potential, -350V; declustering potential -10V. The samples were analysed with an IDA (Information-Dependent Acquisition) method, which can automatically select candidate ions for MS/MS study. The TOF mass range was set from *m/z* 100 to 1000 and the mass range for product ion scan was *m/z* 50 to 1000. The collision energy (CE) was set from 15 to 45eV for more structural information. The mass analyser was calibrated using Taurocholic Acid (2 ng/μL) by direct injection at a flow rate of 5 μL/min. The data were acquired and processed using Analyst QS 2.0 software.

Results and discussion

A qualitative characterization of chemical components in *N. scrphulariiflora* roots was performed on ESI negative ionization mode. Accurate mass data were acquired in the full scan analysis and product ion mass were acquired in IDA (Information-Dependent Acquisition) method. ACQUITY UPLC® HSS T3 column were employed to provide increased chromatographic resolution. In addition, formic acid was introduced into the mobile phase (0.1%) to alleviate the peak tailing and produce better shapes. The acidic conditions did not appear to significantly affect the ionization efficiency of compounds in negative mode. A total of 24 compounds were tentatively characterized and structures of them were shown in **Figure 2**. The total ion current (TIC) profiles was shown in **Figure 1**, accurate mass measurements, retention time, formula, errors for all compounds were summarized in **Table 1** as well as the main product ions observed in MS/MS spectrum.

Optimization of fragmentor voltage

In order to optimize signals and obtain more structural information, different CE values were performed in the MS/MS experiments. For analysis of iridoid glycosides, CE 15 eV was enough to obtain good deprotonated ions and 30 eV was used to obtain information about aglycone skeleton. For phenylethanoid glycosides with disaccharide, CE 30 eV was enough to give good strong deprotonated molecular and 33eV provided the characteristic fragment ions for structure elucidation. For phenylethanoid glycosides with trisaccharide, CE 33 eV was sufficient to provide deprotonated molecular while CE 40 eV provided good structural information as well as good relative abundance of fragment ions. For phenyl glycosides, 30 eV was enough to provide characteristic fragment ions.

Fragmentation patterns of iridoid glycosides

Iridoid display the characteristic framework, in which the cyclopentanoid unit is fused with a dihydropyran ring and a glucose moiety usually attached to the C-1 position [11]. Based on the basic structural skeleton, iridoid glycosides are classified to several types: cyclopentene-type, cyclopentane-type, and epoxytane-type iridoid glycosides [12]. The fragmentation patterns of iridoid glycosides were revealed as follows based on the literature reported.

a. Formate adduct ion $[M+HCOO]^-$ indicated the presence of hydroxyl at C-5 position or methyl ester at C-4 position [13].

b. Neutral loss of H_2O, CO_2, a glucose unit and different benzene substituent groups (such as caffeoyl, vanilloyl) are common characteristic.

c. The glycosidic bond of iridoid glycosides was prone to neutral loss of 162 u. However, the neutral loss will be 180 u if the hydroxyl group was not at the C-8 position or at the carbon directly linked to C-8 [14].

d. Neutral loss of a molecule of methanol (CH_3OH, 32 u) will be occurred when a *β*-hydroxyl group located at C-6 or C-8 position [14].

e. Cyclopentene-type and cyclopentane-type iridoid glycosides were prone to loss of C_2H_4O (44 u) or 3-oxopropanoate (88 u), which corresponded to the isomerization of the hemiacetal group in iridoid aglycone. Common fragmentation ion at *m/z* 101 with the elemental composition $C_4H_5O_3$ was usually observed. These two types of iridoid glycosides can be differentiated on the basis of the relative abundances of fragmentation ions [13].

f. Epoxytane-type iridoid glycosides with different benzene substituent groups substituted to the C-6 position was facile to loss of iridoid (182 u) and loss of noteworthy 114 u, which were resulted from hemiacetal group isomerized to two aldehyde units and cleavage of the rings of the basic skeleton [15].
g. Epoxytane-type iridoid glycosides with different benzene substituent groups substituted to the C-6 position of *β*-glucopyranose were characterized by loss of iridoid aglycone (200 or 202 u) [16].

Iridoid glycosides in *N. scrphulariiflora* roots

According to fragmentation rules and comparison with MS data reported. 12 iridoid glycosides were identified. Most of them were derivatives of catalpol or 3,4-dihydrocatalpol.

Group I

Group I were epoxytane-type iridoid glycosides: C-7 and C-8 positions of iridoid skeleton were linked through an aglycone oxygen bridge, C-6 position of glucose was substituted by hydroxyl derivatives of cinnamic acid (such as vanilloyl and feruloyl).
Compound **1** showed $[M–H]^-$ ion at *m/z* 361 with the elemental composition of $C_{15}H_{21}O_{10}$. In MS/MS spectrum, ion at *m/z* 199 ($C_9H_{12}O_5$) with high abundance indicated loss of a glucose (162 u) from $[M–H]^-$ ion. Ions at *m/z* 181 ($C_9H_9O_4$) and 169 suggested successive loss of water (18 u) and CH_2O (30 u) from ion at *m/z* 199. Based on accurate mass and formulae provided by QTOF-MS and compared with previous literature report, tentative identification given to compound **1** was catalpol [15].
Compound **2** displayed $[M–H]^-$ at *m/z* 513. Intensive ion at *m/z* 167 demonstrated the presence of vanilloyl moiety. Ion at *m/z* 311, loss of 202 u with elemental composition of $C_9H_{14}O_5$ from $[M–H]^-$ ion, indicated the iridoid aglycone. Noteworthy ions at *m/z* 269 $[M–H–C_{11}H_{16}O_6]^-$ and 209 $[M–H–C_{11}H_{16}O_6–C_2H_4O_2]^-$ demonstrated the fragmentation pattern of crossing-ring cleavage of the glucose moiety. According to the literature, compound **2** was tentatively characterized as piscroside A [17]. **Scheme 1** displayed the proposed fragmentation pathway of compound **2**.
Compound **3** gave $[M–H]^-$ ion at *m/z* 523. In its MS/MS spectrum, ions at *m/z* 323 suggested loss of iridoid moiety (200 u, $C_9H_{12}O_5$) from deprotonated ion. It was presumed that the difference of iridoid aglycone between compound **3** and compound **2** was the double bond at C-3 and C-4 positions. Ions at *m/z* 281 and 221 indicated successive loss of 242 u and 60 u through crossing-ring cleavage of the glucose moiety from deprotonated ion. Characteristic ions at *m/z* 179 and 161 supplied the evidence for the existence of caffeoyl moiety. Comparing with previous literature report, tentative identification given to compound **3** was 6′-O-caffeoylcatalpol [18].
Compound **4** showed $[M–H]^-$ ion at *m/z* 509. In analysis of MS/MS spectrum, diagnostic ions at *m/z* 163 and 145 with high abundance served as evidence for the presence of coumaroyl moiety. Ion at *m/z* 307 indicated loss of iridoid moiety from $[M–H]^-$ ion. This allowed us to infer that there was no double bond between C-3 and C-4 positions. Characteristic ions at *m/z* 265 and 205 suggested successive loss of $C_{12}H_{20}O_5$ (244 u) and $C_2H_4O_2$ (60 u) from $[M–H]^-$ ion. According to previous literature report, compound **4** was tentatively identified as piscroside B [17].
Compound **6** gave $[M–H]^-$ at *m/z* 539. A difference of 30 u between pseudo-molecular of compound **4** and **6** suggested the feruloyl moiety at C-6 position of glucose. Diagnostic ions at *m/z* 193 and 175 confirmed the existence of the feruloyl moiety. $[M–H–\text{iridoid}]^-$ ion at *m/z* 337, $[M–H–C_{12}H_{20}O_5]^-$ ion at *m/z* 295 and $[M–H–C_{12}H_{20}O_5–C_2H_4O_2]^-$ ion at *m/z* 235 were observed. Compound **6** was tentatively characterized as 6′-O-ferulyol-3,4-dihydrocatalpol based on fragmentation rules. Compound **6** was potentially novel compound.
Compound **7** exhibited deprotonated ion at *m/z* 507. In MS/MS spectrum, characteristic fragmentation ions at *m/z* 163 and 145 suggested the presence of coumaroyl moiety. $[M–H–\text{iridoid}]^-$ ion at *m/z* 307, $[M–H–C_{12}H_{20}O_5]^-$ ion at *m/z* 265 and $[M–H–C_{12}H_{20}O_5]^-$ ion at *m/z* 205 were obtained. According to the data reported, a tentative identification given to compound **7** was picroside IV [16].
Compound **8** displayed $[M–H]^-$ at *m/z* 537, 30 u higher than that of compound **7**. In analysis of MS/MS spectrum, the fragmentation patterns were quite similar to that of compound **7**. It was presumed that compound **8** bear feruloyl moiety instead of coumaroyl moiety. Diagnostic ion at *m/z* 175 further verified the deduction. Accoring to previous report, compound **8** was tentatively identified as picroside III [16].

Group II

Group II were also epoxytane-type iridoid glycosides: C-7 and C-8 positions of iridoid skeleton were linked through an aglycone oxygen bridge, C-6 position of iridoid was substituted by hydroxyl derivatives of cinnamic acid (vanilloyl, caffeoyl or feruloyl moiety). The difference between group I and II were positions of hydroxyl derivatives substituted. Iridoid glycosides of group II were found to be predominant constituents of *N. scrphulariiflora* roots.
Compound **16** showed $[M–H]^-$ ion at *m/z* 507. In its MS/MS spectrum, diagnostic ions at *m/z* 163 with high abundance indicated the presence of coumaroyl moiety. Ion at *m/z* 345 indicated the characteristic neutral loss of glucose moiety (162 u) from $[M–H]^-$ ion. The significant ion $[M–H–\text{glucose}–114]^-$ at *m/z* 231 suggested loss of $C_5H_6O_3$, which was resulted from two aldehyde units isomerized from hemiacetal group and cleavage of the ring of the basic skeleton. According to above information and rules in literature, compound **16** was tentatively elucidated as specioside [15].
Compound **9** produced $[M–H]^-$ at *m/z* 511. In analysis of MS/MS spectrum, ion at *m/z* 349 indicated the characteristic neutral loss of glucose moiety (162 u) from $[M–H]^-$ ion. The significant ion at *m/z* 235 suggested loss of $C_5H_6O_3$ (114 u) from $[M–H–\text{glucose}]^-$ ion at *m/z* 349. Characteristic ion at *m/z* 167 with higher abundance coupled with ions at *m/z* 152 and 123 demonstrated a vanilloyl moiety. Based on literature, Compound **9** was tentatively identified as picroside II [15].
In the first-order MS of Compound **11**, the $[M–H]^-$ ion at *m/z* 523 as the base peak was observed, in the MS/MS spectrum, product ions at *m/z* 361 $[M–H–\text{glucose}]^-$, 247 $[M–H–\text{glucose}–C_5H_6O_3]^-$ and characteristic ions of caffeoyl moiety at *m/z* 179, 161 were observed. Comparing with literature reported, Compound **11** was tentatively identified as verminoside [15].
Compound **18** exhibited deprotonated ion at *m/z* 537. In analysis of its MS/MS spectrum, $[M–H–\text{glucose}]^-$ ion at *m/z* 375 was 14 u higher than that of compound **11**, it presumed that the existence of feruloyl moiety, ions at *m/z* 193 and 175 confirmed the deduction. The ion at *m/z* 261 was corresponding to the noteworthy loss of 114 u from ion at *m/z* 375. According

to above information and rules in literature, compound **18** was tentatively identified as minecoside [15] and its appropriate fragmentation pathway was proposed in **Scheme 2**.

Group III

Iridoid glycosides of group III characterized as cyclopentane-type iridoid glycosides with no double bond within the five-membered-ring.

Compound **13** exhibited $[M+HCOO]^-$ ions at *m/z* 539 and $[M-H]^-$ ions at *m/z* 493. Ion at *m/z* 345 indicated loss of cinnamoyl moiety from deprotonated ion, characteristic ion at *m/z* 147 confirmed the existence of cinnamoyl moiety. $[M-H-cinnamoyl-162]^-$ ion at *m/z* 183 suggested elimination of a glucose from ion at *m/z* 345. By comparing with the data reported, compound **13** was tentatively identified as harpagoside [19-20]. A fragmentation pattern of compound **13** was proposed in **Scheme 3**.

Phenyl glycosides *in N. scrphulariiflora* roots

Phenyl glycosides were minor in *N. scrphulariiflora* roots. The skeleton of phenyl glycosides is a glucose linked to benzene through glycosidic bond. The C-6 position of glucose is substituted by cinnamoyl or vanilloyl moiety. Breakage of glucosidic linkage was the main fragmentation pathway of phenyl glycosides.

Compound **5** showed deprotonated ion at *m/z* 641. In MS/MS spectrum, ions at *m/z* 479 and 311 demonstrated successive loss of glucose moiety and vanilloyl moiety from deprotonated ion. Characteristic ion at *m/z* 167 with high abundance suggested the existence of vanilloyl moiety. Based on the literature, tentative identification given to compound **5** was scrophenoside D [21]. **Scheme 4** demonstrated the fragmentation pathway of compound **5**.

Compound **21** gave $[M-H]^-$ at *m/z* 443. In its MS/MS spectrum, ion at *m/z* 307 indicated the loss of 4-acetylphenyl (136 u) from $[M-H]^-$ ion. Meanwhile, characteristic ions of coumaroyl moiety at *m/z* 163 and 145 were observed. According to the literature, compound **21** was tentatively identified as scrophenoside B [6].

Compound **22** exhibited $[M-H]^-$ ion at *m/z* 473. In analysis of MS/MS spectrum, diagnostic ions at *m/z* 163 and 145 suggested the presence of coumaroyl moiety. Ion at *m/z* 307 indicated a loss of 166 u from ion at *m/z* 473. It was presumed that 4-acetyl-2-methoxyphenyl substituted to glucose moiety. Comparing with previous literature report, a tentatively identification given to compound **22** was scrophuloside A [7].

Fragmentation patterns of phenylethanoid glycosides

Phenylethanoid glycosides are characterized by a *β*-glucopyranose attached to hydroxyphenylethyl moiety directly. The C-4 or C-6 position of *β*-glucopyranose was usually substituted by hydroxyl derivative of cinnamic acid (such as caffeoyl and feruloyl). Another sugar moiety usually located at the C-2 or C-3 position of *β*-glucopyranose. The main and typical loss are loss of feruloyl moiety (176 u), caffeoyl or hexose moiety (162 u), deoxyhexose moiety (146 u), pentose moiety (132 u) and loss of 42, 32, 18 u, which indicated the presence of acetyl, methoxyl and hydroxyl on aglycone moiety.

Phenylethanoid glycosides *in N. scrphulariiflora* roots

Compound **10** showed the deprotonated molecule at *m/z* 801. In MS/MS spectrum, ion at *m/z* 639 suggested loss of caffeoyl moiety from deprotonated ion, identical ions at *m/z* 179 and 161 with high abundance confirmed the presence of caffeoyl moiety. Ion at *m/z* 315 $[M-H-caffeoyl-162-162]^-$ suggested compound **10** bear glucoses both at C-3 and C-5 positions of central *β*-glucopyranose. Comparing with previous literature report, compound **10** was tentatively characterized as maxoside [22].

Compound **12** and **15** showed the same $[M-H]^-$ at *m/z* 639 and displayed almost same product ions in MS/MS spectrum, which indicated that compound **12** and **15** were isomers with the same elementary composition but different substitution positions. In the MS/MS spectrum, ions at *m/z* 477 and 315 demonstrated successive loss of caffeoyl moiety and glucose moiety from $[M-H]^-$ ion. Intensive ions at *m/z* 179 and 161 served as the evidence for the presence of caffeoyl moiety. Compound **12** and **15** were tentatively identified as plantamajoside and isomer of plantamajoside based on the observed fragment behaviours and data reported [23].

Compound **14** gave deprotonated molecule at *m/z* 477. In MS/MS spectrum, diagnostic ions at *m/z* 179 and 161 with high abundance suggested the presence of caffeoyl moiety. The ion at *m/z* 315 further demonstrated a caffeoyl moiety. Comparing with previous literature report, a tentative identification of compound **14** was calceorioside B [24].

Compound **17** gave $[M-H]^-$ at *m/z* 653. In analysis of MS/MS spectrum, characteristic fragmentation ions at *m/z* 179 and 161 suggested the presence of caffeoyl moiety. Ions at *m/z* 491 $[M-H-162]^-$ and 329 $[M-H-162-162]^-$ indicated successive loss of caffeoyl and glucose moiety from deprotonated ion at *m/z* 653. According to fragmentation rules, compound **17** was characterized as 1-[2-(4-(3,4-dihydroxycinnamoyl)-3-glucosyl)glucosy]ethy-3-hydroxy-4-methoxybenzene, which was potentially novel compound.

Compound **19** displayed $[M-H]^-$ at *m/z* 813. In its MS/MS spectrum, diagnostic ions at *m/z* 193 and 175 with high abundance supplied evidence for the existence of feruloyl moiety. Ions at *m/z* 491 and 329 indicated the successive loss of rhamnose moiety and glucose moiety from $[M-H-feruloyl]^-$ at *m/z* 637. Comparing with previous literature report, a tentative identification of compound **19** was scroside H [25]. The appropriate fragmentation pathway of compound **19** is proposed in **Scheme 5**.

Compound **20** showed $[M-H]^-$ at *m/z* 829. In MS/MS spectrum, diagnostic ions at *m/z* 193 and 175 with high abundance indicated the presence of feruloyl moiety. Ion at *m/z* 667 suggested loss of glucose moiety from $[M-H]^-$ ion. Ion at *m/z* 329 indicated loss of 162 u from $[M-H-glucose-feruloyl]^-$ ion at *m/z* 491. It was presumed that compound **20** bear two glucoses in central *β*-glucopyranose. Based on data reported, compound **20** was tentatively identified as scroside A [26].

Compound **23** gave deprotonated ion at *m/z* 667, 162 u lower than that of compound **20**. In MS/MS spectrum, the fragmentation pathways were quite similar to that of compound **20**. According to previous report, compound **23** was tentatively elucidated as scroside B [26].

Compound **24** exhibited $[M-H]^-$ at *m/z* 651. In MS/MS spectrum, fragmentation ions at *m/z* 193 and 175 with high abundance revealed the existence of feruloyl moiety. Ions at *m/z* 329 indicated the loss of rhamnose moiety from $[M-H-feruloyl]^-$ at *m/z* 475. It was presumed that a methoxyl added to the phenylethyl group of compound **24**. Comparing with

previous literature report, compound **24** was tentatively characterized as martynoside [27].

Conclusions

A reliable and effective method employing HPLC-QTOF-MS/MS was developed for unequivocally identification of chemical components in *N. scrphulariiflora* roots. Based on accurate mass measurement and the characteristic fragmentation, 24 chemical components, including 12 iridoid glysosides, 3 phenyl glycosides and 9 phenylethanoid glycosides were tentatively identified. Among them, 6′-O-ferulyol-3,4-dihydrocatalpol and 1-[2-(4-(3,4-dihydroxycinnamoyl)-3-glucosyl)glucosy]ethy-3-hydroxy-4-met-hoxybenzene were two potentially novel compounds. 6′-O-caffeoylcatalpol and maxoside were characterized in *N. scrphulariiflora* for the first time.
This established HPLC-QTOF-MS/MS method represents a powerful tool to prediction of chemical constituents in a complex plant extract and could be the basis for the comprehensive quality control of *N. scrphulariiflora* roots.

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Notes and references

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Table 1. Identification of chemical components in *N. scrphulariiflora* roots by HPLC-QTOF-MS/MS
Figure 1. Chromatogram of *N. scrphulariiflora* roots analysed by HPLC-TOF-MS in negative ESI mode
Figure 2. Chemical components detected in *N. scrphulariiflora* roots
Scheme 1. Proposed fragmentation pathway of compound **2**
Scheme 2. Proposed fragmentation pathway of compound **18**
Scheme 3. Proposed fragmentation pathway of compound **13**
Scheme 4. Proposed fragmentation pathway of compound **5**
Scheme 5. Proposed fragmentation pathway of compound **19**
See DOI: 10.1039/b000000x/

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Table 1. Identification of chemical components in *N. scrphulariiflora* roots by HPLC-QTOF-MS/MS

No.	tR min	Formula	$[M-H]^-$	Error ppm	CE eV	ESI-MS^n data	Identification
Iridoid glycoside							
1	2.29	$C_{15}H_{22}O_{10}$	361.1126	-2.41	30	MS: 361[M-H] MS^2: 199[M-H-Glc] 181[M-H-Glc-H_2O] 169[M-H-Glc-CH_2O] 151[M-H-Glc-CH_2O-H_2O]	Catalpol
2	27.69	$C_{23}H_{29}O_{13}$	513.1570	-7.43	30	MS: 513[M-H] MS^2: 311[M-H-$C_9H_{14}O_5$] 269[M-H-$C_{11}H_{16}O_6$] 209[M-H-$C_{11}H_{16}O_6$-$C_2H_4O_2$] 167[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$] 152[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$-•CH_3] 123[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$-CO_2]	Piscroside A
3	33.54	$C_{24}H_{27}O_{13}$	523.1450	-0.32	30	MS: 523[M-H] MS^2: 323[M-H-$C_9H_{12}O_5$] 281[M-H-$C_{12}H_{18}O_5$] 221[M-H-$C_{12}H_{18}O_5$-$C_2H_4O_2$] 179[M-H-$C_9H_{12}O_5$-$C_6H_8O_4$] 161[M-H-$C_9H_{12}O_5$-$C_6H_8O_4$-H_2O]	6′-O-caffeoylcatalpol
4	37.24	$C_{24}H_{29}O_{12}$	509.1658	-0.2	30	MS: 509[M-H] MS^2: 307[M-H-$C_9H_{14}O_5$] 265[M-H-$C_{12}H_{20}O_5$] 205[M-H-$C_{12}H_{20}O_5$-$C_2H_4O_2$] 163[M-H- $C_9H_{14}O_5$-$C_6H_8O_4$] 145[M-H- $C_9H_{14}O_5$-$C_6H_8O_4$-H_2O]	Piscroside B
6	41.84	$C_{25}H_{31}O_{13}$	539.1751	-2.53	30	MS: 539[M-H] MS^2: 337[M-H-$C_9H_{14}O_5$] 295[M-H-$C_{12}H_{20}O_5$] 235[M-H-$C_{12}H_{20}O_5$-$C_2H_4O_2$] 193[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$] 175[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$-H_2O]	6′-O-feruloyl-3, 4-dihydrocatalpol
7	43.52	$C_{24}H_{27}O_{12}$	507.1487	-3.05	30	MS: 507[M-H] MS^2: 307[M-H-$C_9H_{12}O_5$] 265[M-H-$C_{12}H_{20}O_5$] 205[M-H-$C_{12}H_{20}O_5$-$C_2H_4O_2$] 163[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$] 145[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$-H_2O]	Picroside IV
8	47.97	$C_{25}H_{29}O_{13}$	537.1563	-8.4	30	MS: 537[M-H] MS^2: 337[M-H-$C_9H_{12}O_5$]	Picroside III

						295[M-H- $C_{12}H_{20}O_5$]	
						235[M-H- $C_{12}H_{20}O_5$-$C_2H_4O_2$]	
						193[M-H-$C_9H_{12}O_5$-$C_6H_8O_4$]	
						175[M-H-$C_9H_{12}O_5$-$C_6H_8O_4$-H_2O]	
9	51.01	$C_{23}H_{27}O_{13}$	511.1463	2.21	35	MS: 511[M-H]	Picroside II
						MS^2: 349[M-H-Glc]	
						235[M-H-Glc-$C_5H_6O_3$]	
						167[M-H-Glc-$C_9H_{10}O_4$]	
						152[M-H-$C_9H_{10}O_4$-$C_6H_8O_4$-•CH_3]	
						123[M-H-Glc-$C_9H_{10}O_4$-CO_2]	
11	54.28	$C_{24}H_{27}O_{13}$	523.1477	4.84	30	MS: 523[M-H]	Verminoside
						MS^2: 361[M-H-Glc]	
						247[M-H-Glc-$C_5H_6O_3$]	
						179[M-H-Glc-$C_9H_{10}O_4$]	
						161[M-H-Glc-$C_9H_{10}O_4$-H_2O]	
						135[M-H-Glc-$C_9H_{10}O_4$-CO_2]	
13	59.27	$C_{24}H_{29}O_{11}$	493.1681	-5.83	30	MS: 493[M-H]	Harpagoside
						539[M + HCOO]	
						MS^2: 363[M-H-Cinnamoyl]	
						345[M-H-Cinnamoyl-H_2O]	
						201[M-H-Cinnamoyl-Glc]	
						183[M-H-Cinnamoyl-H_2O-Glc]	
						147[M-H-$C_{15}H_{22}O_9$]	
16	70.87	$C_{24}H_{27}O_{12}$	507.1502	-0.1	30	MS: 507[M-H]	Specioside
						MS^2: 345[M-H-Glc]	
						231[M-H-Glc-$C_5H_6O_3$]	
						163[M-H-Glc-$C_9H_{10}O_4$]	
						145[M-H-Glc-$C_9H_{10}O_4$-H_2O]	
18	77.92	$C_{25}H_{29}O_{13}$	537.1584	-4.49	30	MS: 537[M-H]	Minecoside
						MS^2: 375[M-H-Glc]	
						261[M-H-Glc-$C_5H_6O_3$]	
						193[M-H-Glc-$C_9H_{10}O_4$]	
						175[M-H-Glc-$C_9H_{10}O_4$-H_2O]	
Phenolic glycosides							
5	38.65	$C_{28}H_{33}O_{17}$	641.1691	-4.17	30	MS: 641[M-H]	Scrophenoside D
						MS^2: 479[M-H-Glc]	
						311[M-H-Glc-$C_8H_8O_4$]	
						167[M-H-Glc-$C_8H_8O_4$-$C_6H_8O_4$]	
						152[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$-•CH_3]	
						123[M-H-$C_9H_{14}O_5$-$C_6H_8O_4$-CO_2]	
21	93.88	$C_{23}H_{24}O_9$	443,1324	-4.07	25	MS: 443[M-H]	Scrophenoside B
						MS^2: 307[M-H-$C_8H_8O_2$]	
						163[M-H-$C_8H_8O_2$-$C_6H_8O_4$]	
						145[M-H-$C_8H_8O_2$-$C_6H_8O_4$-H_2O]	

						135[M-H-$C_{15}H_{17}O_7$]	
22	98.49	$C_{24}H_{25}O_{10}$	473.1472	-0.39	30	MS: 473[M-H] MS^2: 307[M-H-$C_9H_{10}O_3$] 165[M-H-$C_{15}H_{17}O_7$] 163[M-H-$C_9H_{10}O_3$-$C_6H_8O_4$] 145[M-H-$C_9H_{10}O_3$-$C_6H_8O_4$-H_2O]	Scrophuloside A
Phenylethanoid glycosides							
10	53.28	$C_{35}H_{45}O_{21}$	801.2413	-5.03	40	MS: 801[M-H] MS^2: 639[M-H-Caffeoyl] 477[M-H-Caffeoyl-Glc] 315[M-H-Caffeoyl-Glc-Glc] 179[M-H-$C_{26}H_{38}O_{17}$] 161[M-H-$C_{26}H_{38}O_{17}$-H_2O]	Maxoside
12	54.78	$C_{25}H_{35}O_{16}$	639.1921	-0.64	33	MS: 639[M-H] MS^2: 477[M-H-Caffeoyl] 315[M-H- Caffeoyl-Glc] 179[M-H- $C_{20}H_{28}O_{12}$] 161[M-H- $C_{20}H_{28}O_{12}$-H_2O]	Plantamajoside
14	61.65	$C_{23}H_{26}O_{11}$	477.1395	-0.39	33	MS: 477[M-H] MS^2: 315[M-H-Caffeoyl] 179[M-H-$C_{14}H_{19}O_7$] 161[M-H-$C_{14}H_{19}O_7$-H_2O]	CalceoriosideB
15	65.57	$C_{29}H_{35}O_{16}$	639.1938	2.01	33	MS: 639[M-H] MS^2: 477[M-H-Caffeoyl] 315[M-H-Caffeoyl-Glc] 179[M-H-$C_{20}H_{28}O_{12}$] 161[M-H-$C_{20}H_{28}O_{12}$-H_2O]	Isomer of plantamajoside
17	75.42	$C_{30}H_{37}O_{16}$	653.2019	-9.58	35	MS: 653[M-H] MS^2: 491[M-H-Caffeoyl] 329[M-H-Caffeoyl-Glc] 179[M-H-C21H30O12] 161[M-H-C21H30O12-H2O]	1-[2-(4-(3,4-dihydroxyci nnamoyl)-3-glucosyl)u cosy]ethy-3-hydroxy-4-methoxybenzene
19	84.96	$C_{37}H_{49}O_{20}$	813.2877	7.35	40	MS: 813[M-H] MS^2: 637[M-H-Feruloyl] 491[M-H-Feruloyl-Rha] 329[M-H-Feruloyl-Rha-Glc] 175[M-H-$C_{27}H_{40}O_{16}$-H_2O]	Scroside H
20	87.67	$C_{37}H_{49}O_{21}$	829.2789	3.8	40	MS: 829[M-H] MS^2: 667[M-H-Glc] 491[M-H-Glc-Feruloyl] 329[M-H-Glc-Feruloyl-Glc] 193[M-H-$C_{27}H_{40}O_{17}$] 175[M-H-$C_{27}H_{40}O_{17}$-H_2O]	Scroside A
23	90.91	$C_{31}H_{39}O_{16}$	667.2220	-2.71	35	MS: 667[M-H]	Scroside B

24	100.4	$C_{31}H_{39}O_{15}$	651.2309	3.07	33	MS^2: 491[M-H-Feruloyl] 329[M-H-Feruloyl-Glc] 193[M-H-$C_{21}H_{30}O_{12}$] 175[M-H-$C_{21}H_{30}O_{12}$-H_2O] MS: 651[M-H] MS^2: 475[M-H-Feruloyl] 329[M-H-Feruloyl-Rha] 193[M-H-$C_{21}H_{30}O_{11}$] 175[M-H-$C_{21}H_{30}O_{11}$-H_2O]	Martynoside

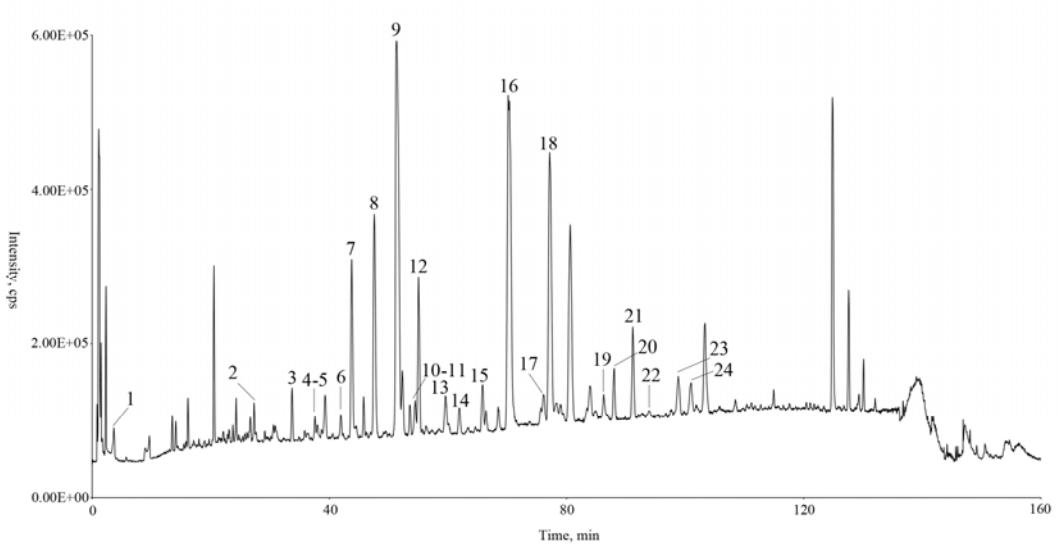


Figure 1. Chromatogram of *N. scrphulariiflora* roots analysed by HPLC-TOF-MS in negative ESI mode

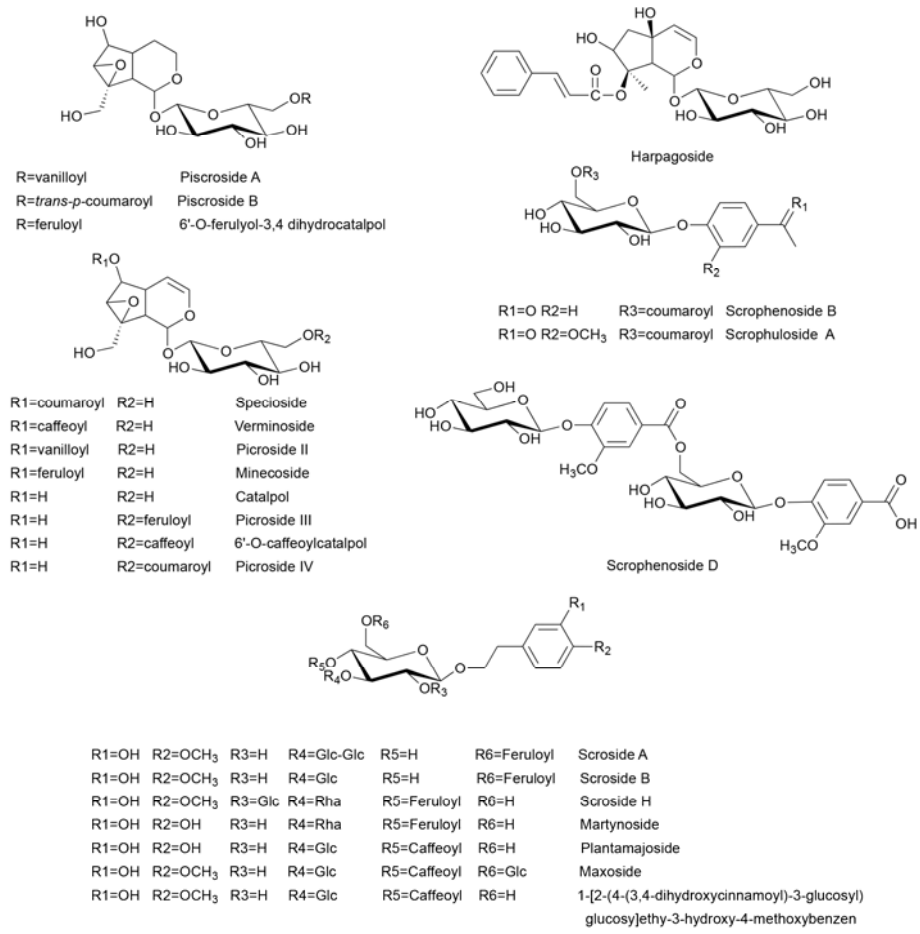
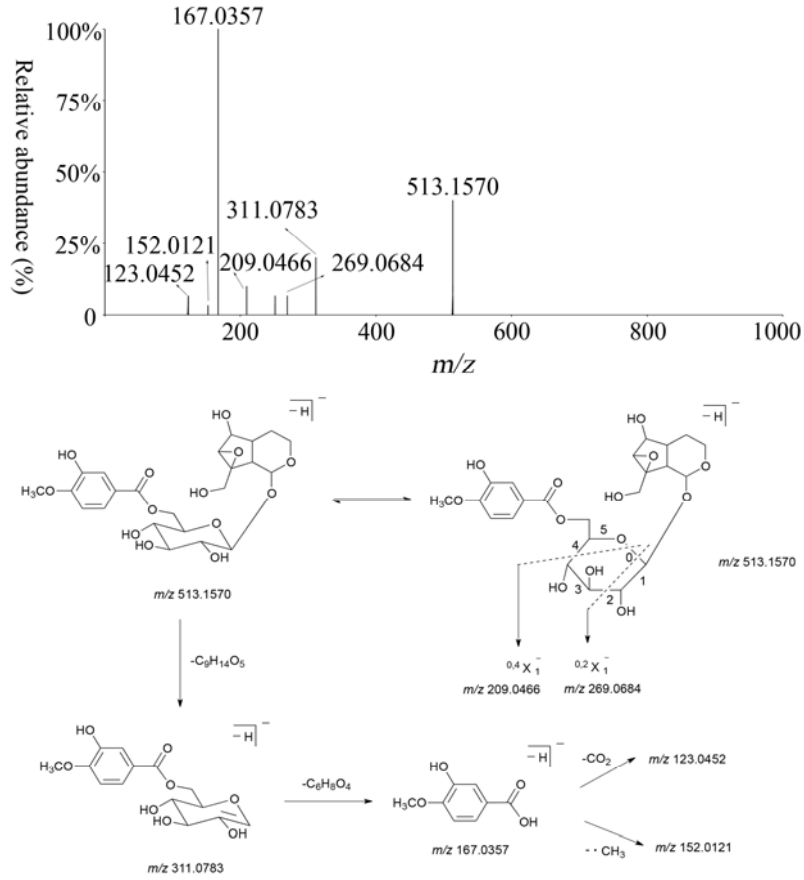
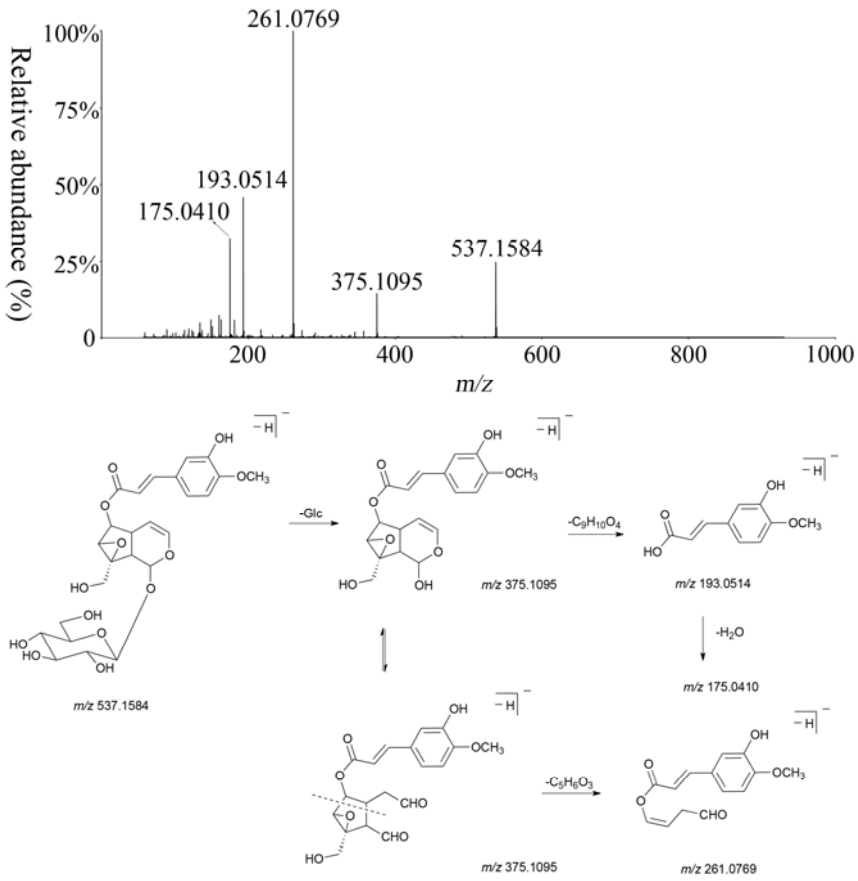


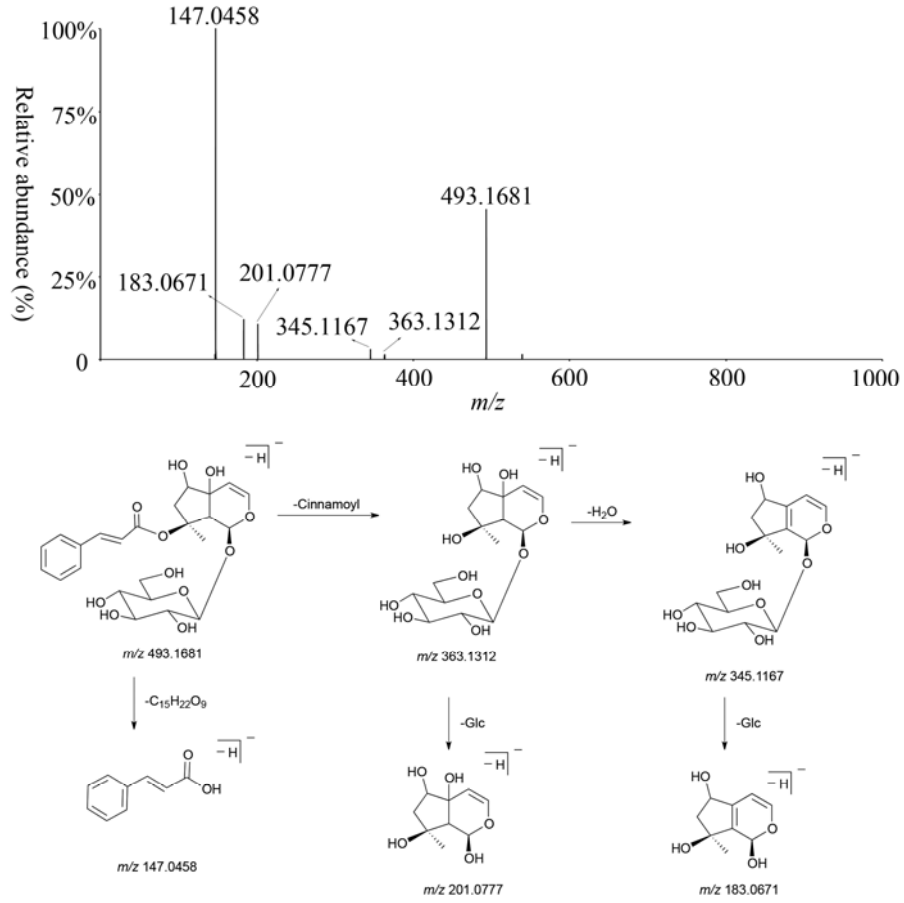
Figure 2. Chemical components detected in *N. scrphulariiflora* roots



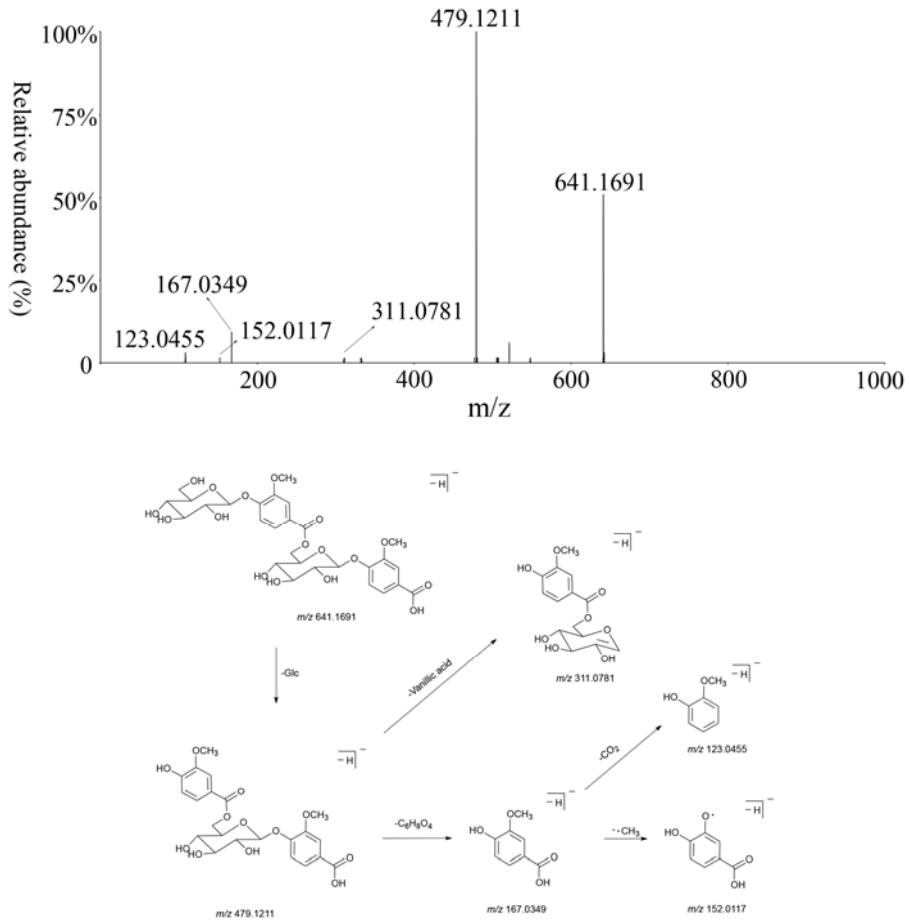
Scheme 1. Proposed fragmentation pathway of compound **2**



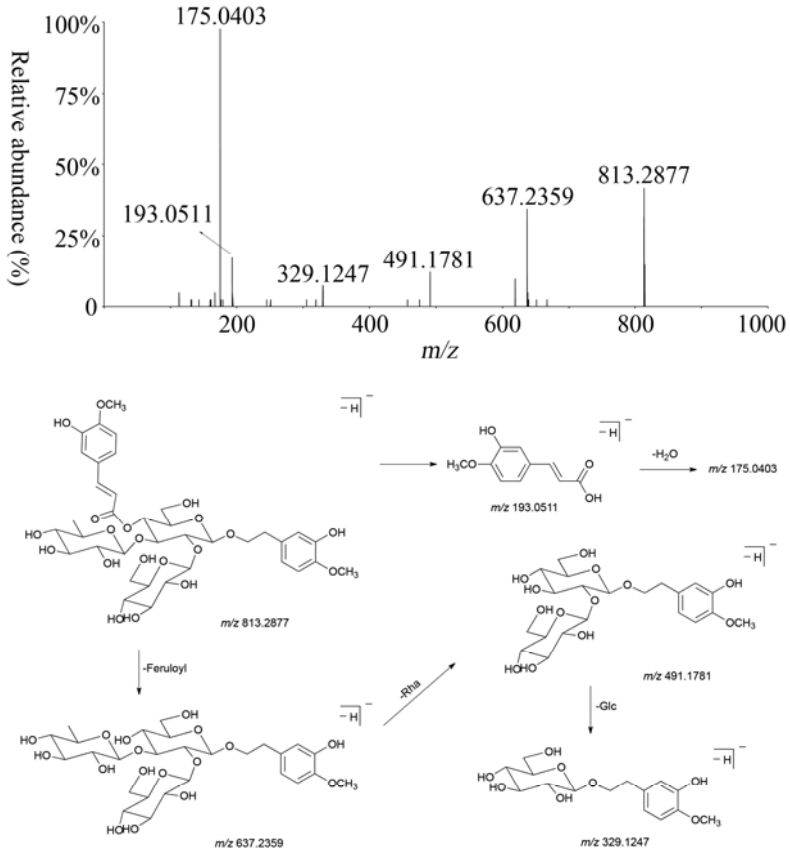
Scheme 2. Proposed fragmentation pathway of compound **18**



Scheme 3. Proposed fragmentation pathway of compound **13**



Scheme 4. Proposed fragmentation pathway of compound **5**



Scheme 5. Proposed fragmentation pathway of compound **19**