Rapid Analysis and Identification of the Main Constituents in Patrinia scabiosaefolia Fisch. by UPLC/Q-TOF-MS/MS

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Summary. Patrinia scabiosaefolia Fisch. (PSF), a well-known traditional Chinese medicine, has been demonstrated to show therapeutic effects on inflammatory bowel disease. In this study, a rapid and sensitive method using ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS) was developed for identification of the major constituents in PSF. The separation analysis was performed on Waters Acquity UPLC system, and the accurate mass of molecules and their fragment ions were determined by Q-TOF-MS. Thirty-one constituents, including triterpenoids, iridoids, flavonoids, and organic acids were detected and tentatively deduced on the basis of their element compositions, tandem mass spectrometry (MS/MS) data, and relevant literatures. Twelve constituents were discovered for the first time in PSF. The results demonstrated that hederagenin-type and oleanolic acid-type saponins were the main constituents of PSF. Our work provides a certain foundation for further quantitation of major chemical constituents and in vivo pharmacokinetic studies of PSF. Moreover, the analytical approach developed herein has proven to be generally applicable for profiling the chemical constituents in traditional Chinese medicines (TCMs) and other complicated mixtures.

Key Words: Patrinia scabiosaefolia Fisch., UPLC/Q-TOF-MS, triterpenoids, iridoids, flavonoids

Introduction

Patrinia scabiosaefolia Fisch. (PSF), a well-known traditional Chinese medicinal plant, is widely distributed in China. Modern pharmacological studies have shown that it possesses antibacterial, antivirus, antitumor, sedative, and protection efficacies of liver, gallbladder, and so on. Our previous study showed that the saponins of PSF could downregulate the expression of inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and...
tumor necrosis factor beta (IL-1β), which are regulated by nuclear factor kappa B (NF-κB), and induce the expression of intestinal metabolic enzymes, resulting in maintained cell barrier integrity [1–3]. These therapeutic properties have been attributed to the major components in PSF.

Although so many beneficial effects have been reported in previous studies, the actual and accurately bioactive components of PSF are still poorly understood. So far, some ingredients have been separated and purified from the rhizome or root of PSF. However, it is not sufficient to identify the bioactive constituents only by the conventional methods. The chemical analysis of PSF as a whole has not yet been elucidated. This is necessary because the effects of TCMs do not simply involve the accumulated effects of individual components, but rather encompass the interactions between various components as a whole. Therefore, qualitative analysis of the total constituents in PSF as a whole provides a more meaningful and specific outcome compared to the individual analysis of each component. In order to provide valuable information for screening of the critical bioactive constituents, it is necessary to develop a specific and sensitive analytical method to identify and characterize the constituents in PSF.

Ultra-performance liquid chromatography with quadruple time-of-flight mass spectrometry (UPLC/Q-TOF-MS) technique is rapid, sensitive in separation, accurate in mass measurement and tandem mass spectrometry (MS/MS), and can be dominant in analysis and quality of complex constituents [4–5]. This paper exploited UPLC/Q-TOF-MS to identify the major components in PSF. To our knowledge, this work is the first study on the chemical components contained in PSF using the methodology developed herein. Thirty-one compounds, including triterpenoids, iridoids, flavonoids, and organic acids, were identified or tentatively characterized by comparing the accurate mass and fragment information with the correlative references data. This work provides a certain foundation for further studies of PSF. More importantly, this novel approach is expected to be widely applied for analyzing other TCMs and complex mixtures.

**Experimental**

**Chemicals and Materials**

Authentic standard such as loganin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China). Acetonitrile was of UPLC grade (Merck, Darmstadt, Germany).
HPLC grade methanol was provided by Honeywell International Inc. (Bur- dick and Jackson, Muskegon, MI, USA). Formic acid was purchased from DIMA (USA). Ultrapure water for the preparation of samples and mobile phase was prepared with PURELAB Ultra GE MK2 water system (ELGA, High Wycombe, UK). Other reagents were of analytical grade.

The sample of PSF (sample no. HX15D03, 3 years old) was collected in October 2013 from Hexiang Medicine Co., Ltd. (Guangdong, China). The original plant of the sample was authenticated by Professor Lai-You Wang (Guangdong Pharmaceutical University). A voucher specimen was deposited in the Institute of Traditional Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou, P.R. China.

The whole plant of the PSF sample was used as experimental material for this study. The air-dried and cut bodies of PSF (500 g) were extracted under reflux with boiling distilled water. The solutions were filtered, pooled, and evaporated under vacuo to obtain the water extract, successively partitioned in a 1:2 ratio with butyl alcohol. The butyl alcohol fraction was absorbed in a polyamide column eluted with water and 95% ethanol. Then, the second water fraction was absorbed in AB-8 macroporous resin column eluted with water and 70% ethanol. The 70% ethanol fraction was the total saponins of PSF. The 70% ethanol extraction was centrifuged at 15,000 rpm for 15 min at 4 °C, and the supernatant was filtered through a 0.20-μm filter; the filtrate was applied for UPLC analysis. The authentic standard loganin was accurately weighed and dissolved in methanol to obtain stock solutions with indicated concentrations. All the stock solutions were stored in the refrigerator at 4 °C until analysis.

**Instrumentation and Analytical Conditions**

The Waters Acquity™ Ultra Performance LC system (Waters Corporation, Milford, USA) was equipped with quaternary pump, vacuum degasser, a cooling autosampler, and a diode-array detector. A UPLC™ BEH C18 column (50 × 2.1 mm, 1.7 μm) was utilized for separation with the column temperature at 30 °C. A binary gradient elution was adopted with mobile phase consisting of (A) 0.1% formic acid in water and (B) acetonitrile: 0–5 min, B 5–15%; 5–7 min, B 15–29%; 7–15 min, B 29–35%; 15–18 min, B 35–50%; and 18–19 min, B 50–100%. The flow rate was set at 0.40 mL min⁻¹. The autosampler was conditioned at 4 °C, and the injection volume was 5 μL.

Waters Micromass Q-TOF micro™ (Waters Co., UK) was equipped
with the Lock Spray and electrospray ionization (ESI) interface operating in negative ion mode and with MassLynx data analysis software. The capillary voltage was set at 3.0 kV, and the cone voltage was set at 5 V. The ion source temperature was set at 100 °C, and desolvation temperature, at 300 °C.

Nitrogen and argon were used for cone and collision gases, respectively. The cone and desolvation gas flows were 60 and 600 L h\(^{-1}\), respectively. The mass spectrometric data was collected in full scan mode with the mass range of \(m/z\) 100–1500, using independent reference lock-mass ions via the Lock Spray interface to ensure mass accuracy and reproducibility. The solution of leucine enkephalin (Sigma Chemical Co.) was used as lock mass, with an \([M−H]^−\) ion of \(m/z\) 554.2615. The MS/MS analysis was performed using a variable collision energy (10–15 eV), which was optimized for each individual constituent. The Lock Spray frequency was set at 10 s. Acquity UPLC/Q-TOF micro system was operated using MassLynx 4.1 software (Waters Co., USA). The accurate mass and composition for the precursor and fragment ions were calculated by MassLynx 4.1.

**Results and Discussion**

The base peak ion (BPI) chromatogram of PSF is shown in Fig. 1. Thirty-one peaks in PSF were detected by using Q-TOF-MS/MS and were identified by comparing accurate molecular weight and elemental compositions of both molecular and some characteristic fragment ions. The elemental compositions of 31 peaks were calculated by the MassLynx software; the results are shown in Table 1.

Among 31 constituents, there are four groups of constituents, such as triterpenoid, iridoids, flavonoids, and organic acids, corresponding to their protonated molecules \([M+HCOO]^−\) and \([M−H]^−\) under the quantitative analysis mode. Four organic acids were discovered for the first time. For example, peaks 4 and 5 are isomers; they have the same molecular ions, i.e., \(m/z\) 707 \([2M−H]^−\) and 353 \([M−H]^−\). The fragmentations at \(m/z\) 179, 191, 135, and 173 via the loss of one molecule of quinic acid, caffeic acid, CO\(_2\), and H\(_2\)O are characteristic fragmentation ways. Therefore, constituents 4 and 5 were identified as chlorogenic acid and cryptochlorogenin acid, respectively [6]. Analogous MS/MS data appeared in constituents 1 and 11.
Table I. MS data of (−) ESI–MS spectra and the identification results of the constituents of PSF

| Peak no. | TR (min) | Identification              | Formula       | Selected ion | Measured mass (m/z) | Error (ppm) | Characteristic ions for confirmation | References |
|---------|----------|-------------------------------|---------------|--------------|---------------------|-------------|-------------------------------------|------------|
| 1       | 0.94     | Quinic acid                   | C₇H₁₂O₆       | M-H         | 191.0560            | 2.1         | 383, 191, 173, 129                  | [6]        |
| 2       | 2.60     | Loganic acid                  | C₁₆H₂₄O₁₀     | M-H         | 375.1283            | -2.1        | 375, 213, 195                       | [7]        |
| 3       | 3.14     | Patriscabroside or its isomer | C₁₆H₂₆O₉      | M+HCOO      | 407.1553            | -1.7        | 407, 361, 199, 161                  | [8]        |
| 4       | 4.29     | Chlorogenic acid              | C₈H₁₅O₉       | M-H         | 353.0873            | 0           | 707, 353, 191, 179, 173, 161, 135   | [6]        |
| 5       | 4.47     | Cryptochlorogenin acid        | C₁₆H₂₄O₉      | M-H         | 353.0874            | 0.6         | 707, 353, 191, 179, 173, 161, 135   | [6]        |
| 6       | 5.74     | Logatin                      | C₁₆H₂₄O₁₀     | M+HCOO      | 435.1503            | -0.9        | 435, 227, 213, 195, 177, 169, 149, 107 | [9]        |
| 7       | 6.65     | Patriscabrol                  | C₁₀H₁₆O₄      | M+H₂O-H     | 217.1072            | -1.8        | 217, 199, 181                       | [9]        |
| 8       | 6.79     | 7-Deoxyloganic acid           | C₁₆H₂₄O₉      | M-H         | 359.1340            | -0.3        | 719, 359, 197, 173, 161             | [10]       |
| 9       | 7.15     | Patrinalloside                | C₂₁H₃₄O₁₁     | M+HCOO      | 507.2077            | -0.2        | 507, 461, 345, 161                  | [11]       |
| 10      | 7.31     | 8-Epideoxyloganic acid        | C₁₆H₂₄O₉      | M-H         | 359.1342            | 0.3         | 719, 359, 197, 173, 161             | [12]       |
| 11      | 7.50     | Dicaffeoyl quinic acid         | C₂₀H₁₅O₁₂     | M-H         | 515.1196            | 1.2         | 1031, 515, 353, 191, 179, 173, 161, 135 | [6]        |
| 12      | 7.71     | Scabioside C                  | C₄₁H₆₇O₁₃     | M+HCOO      | 811.4473            | -0.9        | 811, 765, 603, 471                  | [13]       |
| 13      | 8.08     | Flavovilloside                | C₂₀H₁₄O₁₀     | M-H         | 756.2253            | 1.5         | 755, 609, 463, 301                  | [13]       |
| 14      | 8.29     | Patriscabroside III           | C₂₁H₂₆O₁₁     | M+HCOO      | 507.2079            | 0.2         | 507, 479, 461, 345, 161             | [14]       |
| 15      | 8.50     | α-Hederin                    | C₂₀H₁₆O₁₂     | M+HCOO      | 795.4550            | 2.4         | 795, 749, 603, 471                  | [15]       |
| 16      | 8.88     | Scabioside B                  | C₂₀H₁₆O₁₂     | M+HCOO      | 795.4546            | 1.9         | 795, 749, 587, 455                  | [13]       |
| 17      | 10.42    | Macranthoside A               | C₂₀H₁₆O₁₇     | M+HCOO      | 957.5055            | -0.7        | 957, 911, 779, 633, 471             | [16]       |
| 18      | 11.65    | Patrinia saponin H₃          | C₂₀H₁₆O₁₁     | M-H         | 1381.6652           | 2.9         | 1381, 1235, 1073, 911, 749, 665     | [17]       |
| 19      | 11.96    | Kaleopanax saponin B          | C₂₀H₁₆O₂₆     | M+HCOO      | 1265.6217           | 4.0         | 1265, 1219, 1073, 749, 603, 471     | [17]       |
Table I. (continued)

| Peak no. | TR (min) | Identification | Formula | Selected ion | Measured mass (m/z) | Error (ppm) | Characteristic ions for confirmation | References |
|----------|----------|----------------|---------|--------------|---------------------|------------|-----------------------------------|------------|
| 20       | 12.15    |                | C_{58}H_{94}O_{25} | M+HCOO | 1235.6028 | −2.7 | 1235, 1189, 1027, 617, 455 | [18] |
| 21       | 12.45    | Hederasaponin C | C_{58}H_{94}O_{25} | M+HCOO | 1265.6166 | 0 | 1265, 1219, 1073, 749, 603, 471 | [15] |
| 22       | 12.99    | Cernuoside C    | C_{58}H_{94}O_{25} | M+HCOO | 1119.5634 | 4.2 | 1119, 1073, 911, 633, 471 | [19] |
| 23       | 14.79    | Scabioside E    | C_{58}H_{94}O_{25} | M+HCOO | 1073.5575 | 3.9 | 1073, 1028, 734, 588, 455 | [13] |
| 24       | 15.06    | Scabioside G    | C_{58}H_{94}O_{25} | M−H   | 1322.6420 | −1.7 | 1322, 1160, 1028, 866, 750, 455 | [13] |
| 25       | 15.54    | Scabioside F    | C_{58}H_{94}O_{25} | M−H   | 1161.6050 | −1.1 | 1161, 999, 750, 588, 455 | [13] |
| 26       | 16.20    |                | C_{25}H_{35}O_{17} | M+HCOO | 957.5080 | 2.2 | 957, 779, 717, 455 | [20] |
| 27       | 17.17    | Patriscabratine | C_{25}H_{35}O_{17} | M−H   | 443.1968 | −0.8 | 443, 352, 268, 251, 105 | [21] |
| 28       | 17.37    |                | C_{25}H_{35}O_{17} | M−H   | 1027.5510 | 3.4 | 1027, 895, 749, 617, 455 | [22] |
| 29       | 17.79    | 28-O-glu-β-D-glu-hederagenin ester | C_{25}H_{35}O_{17} | M−H   | 795.4546 | 1.9 | 795, 633, 471, 453 | [23] |
| 30       | 18.95    | Patrinia-plycoside B-II | C_{25}H_{35}O_{17} | M+HCOO | 941.5121 | 2.0 | 941, 895, 733, 587, 455 | [24] |
| 31       | 19.29    | Patrirupin F    | C_{25}H_{35}O_{17} | M−H   | 555.4055 | 2.6 | 555, 537, 481, 325, 293, 205 | [25] |

glu: glucopyranosyl, rha: rhamnopyranosyl, xyl: xylopyranosyl, ara: arabino-
pyranosyl.

aFirst time identified from PSF.
Peaks 2, 3, 6, 7, 8, 10, and 14 were identified as iridoids. For example, peak 6 showed a molecular ion at $m/z$ 435 [M+HCOO]$^-$ in MS spectra and exhibited $m/z$ 227, 213, 195, 177, 161, 169, and 149 ions in the MS$^2$ spectra. The product ion of 227 [M−H−162]$^-$ corresponds to the loss of one molecule of glucose residue. The MS$^2$ fragments were consistent with the following fragmentation pattern: the ion at $m/z$ 213 arose from the loss of a molecule CH$_2$, the ion at $m/z$ 195 was characteristic of the loss of a CH$_3$OH fragment, and the fragment at $m/z$ 177, 169, and 149 might derive from the loss of H$_2$O, COOCH$_2$, and CO from the fragment of the molecule. Pathways of cleavage loganin are shown in Fig. 2. By comparison with the authentic accurate molecular weight and literature data [9], the peak 6 was identified as loganin.

Peaks 9 and 13 were identified as O-glycoside flavonoids. For example, the negative ion ESI-MS spectrum of peak 13 gave a quasimolecular ion [M−H]$^-$ at $m/z$ 755 and three significant fragmentations at $m/z$ 609 [(M−H)−146(deoxyhexose unit)]$^-$, $m/z$ 463 [609−146(deoxyhexose unit)]$^-$, and $m/z$ 301 [463−162(hexose unit)]$^-$.

The MS/MS spectra of peak 13 showed the predominant neutral loss of 146, 146, and 162 Da, correspond-
ing to rhamnopyranosyl and β-D-galactopyranosyl, respectively. The fragmentation ion at \( m/z \) 301 was produced by loss of all linked glucosidic bonds, which was a characteristic fragmentation of flavonoids. As compared with the MS fragments and literatures, peak 13 was plausibly characterized as flavovilloside [13].

Fig. 2. The cleavage pathways of loganin in negative ion mode

The main constituents in PSF were two kinds of saponins, hederagenin-type and oleanolic acid-type. In the MS\(^2\) spectra, aglycone ions \( m/z \) 471 and 455 were finally formed by loss of several glycosidic units, which were the characteristic ions of hederagenin and oleanolic acid, respectively. Thus, peaks 12, 15–26, and 28–31 could be identified as saponins. For example, peak 12 showed a molecular ion at \( m/z \) 811 \([M+HCOO]^-\) and 766 \([M-H]^-\) in MS spectra and exhibited \( m/z \) 604 \([M-H-Glu]^-\) and \( m/z \) 471 \([M-H-Glu-Xyl]^-\) ions in the MS\(^2\) spectra (Fig. 3). The fragmentation ion at \( m/z \) 471 was produced by loss of all linked glucosidic bonds, which was a characteristic fragmentation of hederagenin type saponin [13]. Peak 24 showed a molecular ion at \( m/z \) 1322 \([M-H]^-\) in MS spectra; \( m/z \) 1160 \([M-H-Glu]^-\), \( m/z \) 1028 \([M-H-Glu-Xyl]^-\), \( m/z \) 866 \([M-H-2Glu-Xyl]^-\), \( m/z \) 750 \([M-H-Glu-2Xyl-Rha]^-\), and \( m/z \) 455 \([oleanolic acid]^-\) ions could be detected in the MS\(^2\) spectra, which exhibited a fragmentation pathway corresponding to the loss of glycosidic units (Fig. 4). The fragmentation ion at \( m/z \) 455 corresponds to a characteristic ion of the oleanolic acid moiety [13].
**Fig. 3.** ESI-MS/MS spectrums of peak no. 12 in negative ion mode

**Fig. 4.** ESI-MS/MS spectrums of peak no. 24 in negative ion mode
Conclusion

By analyzing the constituents of PSF based on UPLC–MS technique, a method for rapid analysis of the potential effective constituents in PSF has been established. In this study, 31 constituents were identified by the UPLC/Q-TOF system, including twelve constituents that were discovered for the first time, which enhanced the speed and targeting of bioactive constituents analysis. Over half of them were saponins. Systemic pharmacokinetic investigation and quantitation of the saponins will be carried on in further study. This experiment demonstrates that UPLC/Q-TOF-MS is a powerful and rapid technique in analyzing and discovering the complex constituents in Chinese herbal medicine.

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