Phenolic acids in Fructus Xanthii and determination of contents of total phenolic acids in different species and populations of Xanthium in China

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ABSTRACT  Objective: To study the chemical constituents of Fructus Xanthii and to determine the contents of total phenolic acids (TPA) in fruits of Xanthium from different populations for evaluating the quality of them. Methods: Components in Fructus Xanthii were isolated and purified by various column chromatographies and the contents of TPA were determined by ultraviolet spectrophotometry with chlorogenic acid (CHA) as reference substance. Results: Six caffeoylquinic acids along with caffeic acid and ferulic acid were isolated from Fructus Xanthii. The contents of TPA of the samples collected from 29 populations in China varied from 0.31 % to 1.44 %. Among the samples originated from two species and 1 variety of Xanthium, the contents of TPA in X. sibiricum var. subinere samples with an average of 0.36 % were relatively lower than those in other 2 species. While the content of TPA in Sample 3 collected from Shanghai was 1.44 % and the highest among all the samples, and that in Sample 12 from Xinjian of Jiangxi Province was 0.38 % and the lowest among the X. sibiricum samples. Conclusion: 5-O-cafeoylquinic acid, 1,4-di-O-cafeoylquinic acid and 4,5-di-O-cafeoylquinic acid were isolated from Xanthium plant for the first time. The difference of contents of TPA in samples from different species and different populations in China was relatively significant. Fructus Xanthii in Shanghai and Sanming of Fujian Province were considered high quality if contents of TPA were used.
The genus Xanthium (family Compositae) is represented by 25 species in the world and 3 species and 1 variety in China\(^1\), which are Xanthium sibiricum Patr. (Xanthium strumarium L.), X. mongolicum Kitag., X. inaequilateral DC., and X. sibiricum var. subinerme (Winkl.) Widder. Xanthium species have been used as traditional herb medicines for a long history in oriental countries. Xanthium sibiricum is the principle species abundantly found throughout China, and its fruits (Fructus Xanthii) are used in China for the treatment of nasal sinusitis, headache caused by wind-cold, urticaria and arthritis\(^2\). In continuation of research on biologically active compounds, we conducted the study on X. sibiricum and obtained 8 phenolic acids from n-butanol fraction. Pharmacological studies revealed that phenolic acids were the main active components and the reports from some publications showed that these naturally occurring phenolic acids had various pharmacological properties and could be used to act as cholagogues, stomach stimulants, and immuno-stimulants, as well as anti-inflammatory, antibacterial, and antifungal agents\(^3,4\). In order to utilize this crude drug more reasonably and scientifically, we determined the contents of total phenolic acids (TPA) in 2 species and 1 variety Xanthium samples collected from 29 populations in China by ultraviolet (UV) spectrophotometry\(^5\).

1 MATERIALS

1.1 Apparatus

UV analysis was operated on Shimadzu UV-2550 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were operated on a Bruker DRX-500 spectrometer at 500 MHz for 1H-NMR and 125 MHz for 13C-NMR. Chemical shift was expressed in δ values with reference to tetramethyl-silane (TMS) as internal standard, and coupling constants (\(J\)) were given in Hz. Electron ionization mass spectrometry (EI-MS) was recorded on a Varian MAT-212 mass spectrometer and HRESI on a Q-TOF micro mass spectrometer. Melting point was measured on a RY-2 melting point apparatus that was uncorrected. Infra-red (IR) spectrum was recorded on a Bruker Vector22 spectrometer with KBr pellet.

1.2 Chemicals and drugs

Column chromatography was performed on silica gel (200-300 mesh, Yantai, China), silica gel H (10-40 μm, Yantai, China), and sephadex LH-20 (Pharmacia); Thin-layer chromatography (TLC) analysis was run on HSGF254 precoated silica gel plates (10-40 μm, Yantai, China).

Chemicals are all AR-grade and purchased from Shanghai Chemical Reagent Co., Ltd (Shanghai, China); Distilled deionized water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA); Chlorogenic acid (CHA) was prepared in our lab and its purity was determined to be over 98% by HPLC analysis.

1.3 Plant materials

The ripe fruits of Xanthium sibiricum were collected from a local research farm in Sunqiao town, Shanghai, China in November 2003 and authenticated by Prof. Han-Chen ZHENG, Second Military Medical University. A total of 29 samples of the wild herbs of Xanthium were collected from 19 provinces of China in 2003 and 2004 (Table 1). The voucher specimens of these plants were deposited at the Herbarium of Department of Pharmacognosy, Second Military Medical University, Shanghai, China.

2 METHODS AND RESULTS

2.1 Extraction, isolation and identification

Dried fruits of X. sibiricum (20 kg) were ground and extracted with 75% aqueous ethanol by reflux. The solvent was evaporated under vacuum to afford 1 100 g crude extract (yield, 5.5%). Then the extract was suspended in water and partitioned with petroleum ether, chloroform, ethyl acetate and aqua-saturated n-butanol successively.
Each fraction was evaporated under vacuum to yield the residues of petroleum ether fraction 55 g (5.0%), chloroform fraction 60 g (5.4%), ethyl acetate fraction 60.5 g (5.5%), n-butanol fraction 200 g (18.2%) and aqueous fraction 725 g (65.9%) respectively. The n-butanol fraction (170 g) was subjected to silica gel column chromatography and eluted with ethyl acetate-methanol (30: 1 to 1: 5) to obtain 5 subfractions. Then the subfractions were purified by repeated silica gel column and Sephadex LH-20 chromatography to obtain 8 phenolic acid compounds (Compound 1-8), and their structures were identified by a combination of spectral methods (UV, IR, MS and NMR), see Figure 1 and Table 2.

2.2 Determination of contents of TPA

2.2.1 Sample preparation procedures

The fruits of 29 populations were dried on the laboratory bench at 20-22 °C for 1-2 weeks and pulverized, then the powder was screened through 180 μm sieves. Fine powder (100 mg) was accurately weighed, and 50 ml of 50% methanol was added and the mixture was weighed again. Then, the powder was extracted by reflux in a 85 °C water bath for 2 hours. After cooling, 50% methanol was added to make up to the initial weight. The supernatant fluid was filtered and the filtrate served for analysis.

![Chemical structure of isolated compounds](image-url)
2.2.2 Selection of detecting wavelength

Take the reference solution and Sample 3 solution to scan at 200-400 nm and maximum absorption were obtained at 326.5 nm and 327.5 nm respectively. Then we selected 327 nm as the detecting wavelength.

2.2.3 Calibration curves and linear correlation

CHA 4.4 mg was dissolved in 50% methanol in a 10 ml volumetric flask and diluted to volume (0.44 mg ml) 100, 200, 400, 600, 800 and 100 μl of the above stock solution were each transferred to 10 ml volumetric flask and 50% methanol was added to volume for analysis. Take concentration of above solutions as X-axis and the absorbance at 327 nm as Y-axis to obtain calibration curves and calibration equation (Y = 0.042 38X + 0.010 23, r = 0.997 72). CHA showed a fine linear correlation within linear range of 4.4-44 μg/ml.

2.2.4 Precision, repeatability, stability and recovery

The precision was determined on the CHA solution selected from previous prepared stock solution (17.6 μg/ml), 3 ml of which was taken for analysis for 6 times. The relative standard deviation (RSD, %) values of CHA was 0.55% (n = 6), which showed a good precision.

In order to test the repeatability, five copies of Sample 3 were prepared with reference to sample preparation procedures. And the RSD (%) was 1.21% (n = 5). The above data showed that the analytical method had a good repeatability.

Stability experiments were also done. About 100 mg powdered Sample 3 was taken for analysis according to sample preparation procedures. The contents of TPA were determined at 0, 4, 8, 12, 24 and 48 h. The RSD value was calculated as 0.71% (n = 6). The recovery experiments were carried out to evaluate the accuracy of the method. Five copies of Sample 3 of the same weight (about 50 mg) were added with CHA of the same level (1 mg) and 5 sample solutions were prepared according to sample preparation procedures. Take each of them for analysis and calculate average recovery (%) as 99.78% and the RSD was 1.89% (n = 5), see Table 3.

2.2.5 Determination of contents of TPA in fruits of Xanthium from different populations

The 29 samples from different populations in China were prepared according to sample preparation procedures. The TPA of the 29 samples were determined at 327 nm and calculated according to the calibration equation and the results were summarized in Table 1.

### Table 2: Descriptions of chemical structure of isolated compounds

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>R₆</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3-O-cafeoylquinic acid, chlorogenic acid</td>
<td>H</td>
<td>H</td>
<td>Caffeoyl</td>
<td>H</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5- O-cafeoylquinic acid</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Caffeoyl</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1, 5-di- O-cafeoylquinic acid</td>
<td>H</td>
<td>Caffeoyl</td>
<td>H</td>
<td>H</td>
<td>Caffeoyl</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1, 4-di- O-cafeoylquinic acid</td>
<td>H</td>
<td>Caffeoyl</td>
<td>H</td>
<td>Caffeoyl</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4, 5-di- O-cafeoylquinic acid</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Caffeoyl</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1, 3, 5-tri- O-cafeoylquinic acid</td>
<td>Caffeoyl</td>
<td>H</td>
<td>Caffeoyl</td>
<td>H</td>
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<tr>
<td>7</td>
<td>Caffeic acid</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>8</td>
<td>Ferulic acid</td>
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### Table 3: Recovery of chlorogenic acid in Fructus Xanthii

<table>
<thead>
<tr>
<th>Added (mg)</th>
<th>Found (mg)</th>
<th>Recovery (%)</th>
<th>Mean (%)</th>
<th>RSD (%)</th>
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<td>1.10</td>
<td>1.08</td>
<td>98.2</td>
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</tr>
<tr>
<td>1.08</td>
<td>1.06</td>
<td>98.1</td>
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<td>1.20</td>
<td>102.5</td>
<td>99.78</td>
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<tr>
<td>1.06</td>
<td>1.07</td>
<td>100.9</td>
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</tr>
<tr>
<td>1.18</td>
<td>1.17</td>
<td>99.2</td>
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</table>

3 DISCUSSION

We isolated 8 phenolic acids from fruits of Xanthium sibiricum Patr., 6 of which were caffeoylquinic acids. These caffeoylquinic acids include mono-, di-, and tricaffeoylquinic acid derivatives which were considered to have various pharmaceutical properties, such as chlorogenic acid. Previ-
ous research of our lab also showed that the n-BuOH fraction of Fructus Xanthii produced significant anti-inflammatory, analgesic and anti-allergic activities (another paper reported). Among the 8 compounds, 5- O-caffeoylquinic acid, 1, 4-di- O-caffeoylquinic acid and 4, 5-di- O-caffeoylquinic acid were isolated from Xanthium plant for the first time.

The fruits collected from Shenzhen, Guangdong Province were morphologically different when compared with the species that have been reported in China and it remained to be authenticated and further studied.

UV spectrophotometry was used in our research, which was proved to be simple and effective. It could be applied to determine the contents of phenolic acids. The samples were collected from 19 provinces in China and originated from 2 species and 1 variety of Xanthium. The contents of TPA of the samples varied from 0.31% to 1.44%. Among the samples of 2 species and 1 variety of Xanthium, the contents of TPA in X. sibiricum var. subinerme samples from Beijing, Renqiu (Hebei Province), Xi'an (Shaanxi Province) and Chifeng (the Inner Mongolia Autonomous Region) had the average value of 0.36% and were relatively lower than that in others. The contents of TPA in 3 X. mongolicum samples from Yantai (Shandong Province), Changsha (Hunan Province) and Guilin (Guangxi Autonomous Region) varied from 0.51% to 0.62% with the average of 0.57% and the difference among them was insignificant. While contents of TPA in 21 samples of X. sibiricum, the only Chinese Pharmacopoeia indexed species, showed significant difference. The contents of TPA in Sample 3 and Sample 27 collected from Shanghai and Sanming (Fujian Province) were 1.44% and 1.41% respectively, much higher than the other samples. However the TPA content in Sample 12 from Xinjian (Jiangxi Province) was 0.38% and the lowest among the X. sibiricum samples. As a result, Fructus Xanthii in Shanghai and Sanming were considered high quality if the contents of TPA were used as chemical reference for quality evaluating. These data suggest that the inter- and intra- species variation of Xanthium plant is worth further studying and the quality control of this crude drug is extremely necessary for clinical utilization.

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REFERENCES


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