Preparative Separation and Purification of the Total Flavonoids in *Scorzonera austriaca* with Macroporous Resins

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Abstract: The use of macroporous resins for the separation and purification of total flavonoids to obtain high-purity total flavonoids from *Scorzonera austriaca* was studied. The optimal conditions for separation and purification of total flavonoids in *S. austriaca* with macroporous resins were as follows: D4020 resin columns were loaded with crude flavonoid extract solution, and after reaching adsorptive saturation, the columns were eluted successively with 5 bed volumes (BV) of water, 5 BV of 5% (v/v) aqueous ethanol and 5 BV of 30% (v/v) aqueous ethanol at an elution flow rate of 2 BV h⁻¹. Total flavonoids were obtained from the 30% aqueous ethanol eluate by vacuum distillation recovery. The content of flavonoid compounds in the total flavonoids was 93.5%, which represents an improvement by about 150%. In addition, five flavonoid compounds in the product were identified as 2′′-O-β-D-xylopyranosyl isoorientin, 6-C-α-L-arabopyranosyl orientin, orientin, isoorientin and vitexin by LC-ESI-MS analysis and internal standard methods. The results in this study could represent a method for the large-scale production of total flavonoids from *S. austriaca*.

Keywords: *Scorzonera austriaca*; flavonoids; macroporous resins

1. Introduction

*Scorzonera austriaca* Wild, a perennial herb of the *Compositae*, is widely distributed in the northeast and northwest regions in China, being especially abundant in Jilin Province. It has been widely used as a traditional herbal medicine for curing fevers, carbuncles and mastitis [1]. We have proved that the total flavonoids from *S. austriaca* have hepatoprotective effects and inhibitory effects on hepatitis B virus [2–4], and thus the total flavonoids from *S. austriaca* have been selected as a drug candidate for treating hepatitis B by the National Important Special Foundation of the New Drug Development of China (No. 2013ZX09103002-007). Thus, an efficient method for the separation of the total flavonoids from *S. austriaca* is needed.

To date many methods for flavonoid purification have been developed. These include high speed counter-current chromatography [5], solvent extraction, agarose gel media [6], supercritical carbon dioxide extraction [7], aqueous two-phase flotation and molecule imprinted solid phase extraction [8,9]. However, some of these methods suffer from the shortcoming of needing specific costly instruments, consuming a large amount of organic solvents, low throughput, or being unsuitable on an industrial scale. Alternatively, macroporous resin adsorption technology is an efficient method to prepare bioactive constituents with many advantages, such as a large specific surface area, less consumption of organic solvents, low cost, good mechanical properties and easy regeneration, and is favorable
for industrial applications [10]. Recently, separation methods based on macroporous resins have been applied more and more in laboratories and related industries for obtaining bioactive natural products from *Olea europaea* [11], *Rabdosia serra* [12], *Dryopteris fragrans* [13], pigeon pea roots [14], *Siraitia grosvenorii* [15], and *Canarium album* [16].

However, there are no reports on using macroporous resins to separate the total flavonoids from *S. austriaca* till now, so the aim of this study was to develop an efficient method for the preparative separation and purification of total flavonoids from *S. austriaca* with the optimal resin and separation conditions. The information in this study is significant in the selection of adsorption resins and for separation and purification of total flavonoids from *S. austriaca* in particular or other herbal materials in general.

### 2. Results and Discussion

The following equations were used to quantify the capacities of adsorption and desorption as well as the desorption ratio:

\[
\begin{align*}
Q_a &= (C_0 - C_e)V_i/W \\
Q_d &= C_dV_d/W \\
R_d(\%) &= Q_d/Q_a \times 100\%
\end{align*}
\]

where \(Q_a\) is the adsorption capacity at adsorption equilibrium (mg·g\(^{-1}\) dry resin); \(Q_d\) is the desorption capacity after adsorption equilibrium (mg·g\(^{-1}\) dry resin); \(R_d\) is the desorption ratio (%); \(C_0\) and \(C_e\) are the initial and equilibrium concentrations of total flavonoids in the solution, respectively (mg·mL\(^{-1}\)); \(C_d\) is the concentration of total flavonoids in the desorption solution (mg·mL\(^{-1}\)); \(V_i\) is the volume of the initial sample solution (mL); \(V_d\) is the volume of the desorption solution (mL); \(W\) is the weight of resin (g).

#### 2.1. Screening of Macroporous Resin

A 1.40 mg·mL\(^{-1}\) crude flavonoid extract solution from *Scorzonera austriaca* was used as the test sample. Adsorption and desorption properties of five macroporous resins for total flavonoids were investigated. The results, shown in Table 1, indicated that among the five resins, the D4020 resin possessed a comparatively high adsorption capacity \(Q_a\) (38.7 mg·g\(^{-1}\) dry resin), desorption capacity \(Q_d\) (34.8 mg·g\(^{-1}\) dry resin) and desorption ratio (90.1%). The adsorption kinetics curves for total flavonoids on the five different resins were also obtained (Figure 1). From what can be seen in Figure 1, for all the five different resins, the adsorption capacity of total flavonoids increased with the adsorption time, increasing rapidly in the first 4 h and reaching equilibrium at about 6 h. For total flavonoids in the crude flavonoid extracts, all the five different resins have about the same rate of adsorption. In the comprehensive consideration of the adsorption capacity, desorption capacity, ratio of desorption and rate of adsorption, D4020 resin was fit to separate and purify total flavonoids from the crude flavonoid extracts, and in the following study, D4020 resin was chosen for further experiments.

#### Table 1. Adsorption and desorption properties of five macroporous resins for total flavonoids in the crude flavonoid extracts of *Scorzonera austriaca*.

| Trade Name | \(Q_a\) (mg·g\(^{-1}\)) | \(Q_d\) (mg·g\(^{-1}\)) | \(R_d\) (%) |
|------------|----------------|----------------|----------|
| D101       | 26.4 ± 2.6     | 20.7 ± 2.1     | 78.4 ± 2.3 |
| D4020      | 38.7 ± 2.1     | 34.8 ± 3.0     | 90.1 ± 2.6 |
| NKA-9      | 28.1 ± 3.5     | 25.1 ± 3.1     | 89.4 ± 3.3 |
| X-5        | 22.8 ± 1.7     | 15.6 ± 1.9     | 68.5 ± 2.5 |
| AB-8       | 26.2 ± 2.3     | 23.0 ± 2.6     | 87.7 ± 4.3 |

\(a\) Means of three determinations ± SD.
2.2. Maximum Adsorption Capacity of the Selected Resin

The maximum adsorption capacity of D4020 resin for total flavonoids was obtained through the equilibrium adsorption curve at the different initial concentrations of the crude flavonoid extracts, 0.5, 1.0, 1.5, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 10.0, 20.0 and 40.0 mg·mL⁻¹, respectively. As shown in Figure 2, the adsorption capacity of total flavonoids increased with the initial concentration, and reached the saturation plateau when the initial concentration of the crude flavonoid extracts was 10.00 mg·mL⁻¹ and the maximum adsorption capacity was 192.1 mg·g⁻¹, which indicated that in order to get maximum adsorption capacity, the initial concentration of the crude flavonoid extracts should be equal to or greater than 10.00 mg·mL⁻¹. In the following dynamic adsorption and desorption study, the initial concentration of crude flavonoid extracts was chosen as 20.0 mg·mL⁻¹.

2.3. Effect of Ethanol Concentration on Ratio of Desorption in Static Desorption

In order to choose proper desorption solutions, a sample was desorbed successively with ethanol-water (5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 70%, 95%, v/v) solutions, and the results were showed in Figure 3. At the range of ethanol concentrations from 5% to 30%, the ratios of desorption were higher than other concentrations, and more than 80% of total flavonoids were...
was selected as the proper desorption flow rate in consideration of the lower volume consumption. 

The flow rates investigated in this test were 2, 3 and 4 BV \(\cdot\) h\(^{-1}\), and an ethanol-water solution (40\%, \(v/v\)) was used to elute total flavonoids, with the results shown in Figure 4. Although the elution volume varied little with increasing the flow rate, total flavonoids were totally desorbed with 5 BV, 6 BV and 7 BV of elution volume at the flow rate of 2, 3 and 4 BV \(\cdot\) h\(^{-1}\), respectively. Thus, 2 BV \(\cdot\) h\(^{-1}\) was selected as the proper desorption flow rate in consideration of the lower volume consumption and high efficiency in the dynamic desorption experiments.

**2.4. Desorption Flow Rate in Dynamic Desorption**

The effects of eluate flow rate on ratio of desorption with the ethanol-water (40\%, \(v/v\)) solution in dynamic adsorption.
2.5. Gradient Elution in Dynamic Adsorption and Desorption

On basis of the above experiments, gradient elution test was conducted under the following conditions: The concentration of the crude flavonoid extracts in sample solutions was 20 mg·mL⁻¹ for the adsorption process; in desorption process, the flow rate was 2 BV·h⁻¹ and the eluents were ethanol-water solutions (5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 60%, v/v). Gradient elution has separation effects for different kinds of chemical constituents, TLC can be used to detect the separation effects according the spot color, and thus all the eluates were analyzed by TLC. The volumes of eluents needed in the gradient elution and TLC results of eluates were shown in Table 2. As shown in Table 2, the spots from the eluates of 10%–30% aqueous ethanol (v/v) were yellow, the spots from the eluates of water and 5% aqueous ethanol (v/v) were purple or brown, and the spots from the eluates of above 30% aqueous ethanol (v/v) were brown. In our previous study, we found that purple spots were from chlorogenic acid and its derivatives, the brown spots were from terpenoid compounds, and only yellow spots were from flavonoid compounds. Those indicated that the elution with water and 5% (v/v) aqueous ethanol can remove the chlorogenic acid and part of its derivatives from the crude flavonoid extracts, then flavonoid compounds can be obtained by eluting with 10%–30% (v/v) aqueous ethanol, and last the elution solvents above 30% (v/v) aqueous ethanol were used to clean the resin. In conclusion, the optimal gradient elution is to elute successively with 5 BV of water, 5 BV of 5% (v/v) aqueous ethanol solution and 5 BV of 30% (v/v) aqueous ethanol solution, and the total flavonoids were obtained from the eluate of 30% (v/v) aqueous ethanol solution.

Table 2. The volumes of eluents needed in the gradient elution and TLC results of eluates.

| Eluate     | Water | 5% | 10% | 15% | 20% | 25% | 30% | 35% | 40% | 60% |
|------------|-------|----|-----|-----|-----|-----|-----|-----|-----|-----|
| Volume needed (BV) | 5     | 5  | 5   | 4   | 5   | 4   | 5   | 3   | 2   | 2   |
| Colour of TLC spots | purple, brown | yellow | yellow | yellow | yellow | brown | brown |

2.6. Preparation Method Validation

On basis of the above experiments, the appropriate preparation and purification process of total flavonoids from crude flavonoid extracts of *S. austriaca* on D4020 resin was as follows: the pretreated D4020 resin column was loaded with the crude flavonoid extract solution at the initial concentration of 20 mg·mL⁻¹. After reaching adsorptive saturation, the column was eluted successively with 5 BV of water, 5 BV of 5% (v/v) aqueous ethanol and 5 BV of 30% (v/v) aqueous ethanol, and the elute flow rate is 2 BV·h⁻¹. The total flavonoids were obtained from the eluate of 30% (v/v) aqueous ethanol solution by vacuum distillation recovery.

Six total flavonoids samples were prepared from the same sample of the crude flavonoid extracts of *S. austriaca* by this process and their contents and recovery yields were determined by the ultraviolet-visible spectrophotometry method (Table 3). As shown in Table 3, the total contents of total flavonoids in final products were more than 90%, which had been improved about 150% after the preparation and purification process procedure, compared to the content of total flavonoids in the crude flavonoid extracts (62.1%). And the RSD (%) was 1.85, indicating that this method is stable and feasible.

Table 3. Verified results of preparation and purification tests by D4020 macroporous resin.

| No. | 1       | 2       | 3       | 4       | 5       | 6       | mean   | RSD/% |
|-----|---------|---------|---------|---------|---------|---------|--------|-------|
|     | Recovery yield (%) | 63.40   | 65.55   | 64.46   | 62.50   | 61.72   | 63.83   | 63.58 | 1.37  |
|     | Content (%)       | 93.68   | 90.95   | 92.54   | 94.28   | 96.50   | 93.18   | 93.52 | 1.85  |

a Recovery yield = weight of final product/weight of crude flavonoid extracts × 100.
2.7. HPLC Analysis of the Final Products and Crude Flavonoid Extracts of Scorzonera austriaca

Figures 5–8 illustrate the HPLC chromatograms of the final products and crude flavonoid extracts of S. austriaca and the structural assignments of the six peaks are given in Table 4. Structural elucidations were done by LC-ESI-MS analysis and by using internal standard methods with available reference standards. As shown in Figure 5, six peaks with retention times of 19.08, 30.67, 31.54, 33.15, 34.99 and 41.81 min in the HPLC chromatogram of crude flavonoid extracts were identified as chlorogenic acid [17], 2′″-O-β-D-xylopyranosyl isoorientin [18], 6-C-α-L-arabopyranosyl orientin [18], orientin [19], isoorientin [19] and vitexin [18], and the content of orientin was 9.5%. However, in the HPLC chromatogram of the final products (Figure 6), the chlorogenic acid peak with a retention time of 19.08 min disappeared, the peak area of all other peaks identified increased, and the content of orientin increased to 13.4%, which suggested that this preparation and purification process can remove the chlorogenic acid and enhance the content of total flavonoids. In the HPLC chromatograms recorded at detection wavelength 207 nm (Figures 7 and 8), the terpenoid peaks disappeared in the HPLC chromatogram of the final products, which indicated that this preparation and purification process can also remove terpenoids.

**Figure 5.** HPLC chromatogram of the crude flavonoid extracts (λ = 337 nm). The peaks were identified as chlorogenic acid (I), 2′″-O-β-D-xylopyranosyl isoorientin (II), 6-C-α-L-arabopyranosyl orientin (III), orientin (IV), isoorientin (V), and vitexin (VI).

**Figure 6.** HPLC chromatogram of the final product (λ = 337 nm). Comparing with Figure 5, the peak of chlorogenic acid (I) did not appeared.
Figure 7. HPLC chromatogram of crude flavonoid extracts ($\lambda = 207$ nm). The broad peak with retention time of 55.47 min was terpenoids.

Figure 8. HPLC chromatogram of final products ($\lambda = 207$ nm). Comparing with Figure 7, the broad peak with a retention time of 55.47 min disappeared.

Table 4. Peak assignment for the compounds in the final product and the crude flavonoid extracts of *Scorzonera austriaca*.

| Peak No. | Retention Time (min) | HRMS (m/z) [M + H]$^+$ | MS (m/z) Calculated | Structural Identification | Reference |
|----------|----------------------|--------------------------|---------------------|--------------------------|-----------|
| I        | 19.08                | 355.1027                 | 355.1024            | Chlorogenic acid          | [17]      |
| II       | 30.67                | 581.1527                 | 581.1501            | 2'"-O-β-D-xylopyranosyl isoorientin | [18]      |
| III      | 31.54                | 565.1580                 | 565.1552            | 6-C-α-L-arabipyranosyl orientin | [18]      |
| IV       | 33.15                | 449.1089                 | 449.1078            | Orientin                 | [19]      |
| V        | 34.99                | 449.1093                 | 449.1078            | Isoorientin              | [19]      |
| VI       | 41.81                | 433.1156                 | 433.1129            | Vitexin                  | [18]      |

3. Experimental Section

3.1. Materials

*Scorzonera austriaca* herbs were collected in Siping District in Jilin Province, China, and identified by Prof. Jing-min Zhang of School of Pharmaceutical Sciences, Jilin University, Changchun, China. Distilled water was purchased from Hangzhou Wahaha Group Co., Ltd. (Hongzhou, China). Acetonitrile of chromatographic grade for HPLC was purchased from Fisher Scientific (Fair Lawn, NJ, USA).
Chlorogenic acid was purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 2′,3′-O-β-D-Xylopyranosyl isoorientin, 6-C-α-L-arabipyranosyl orientin, orientin, isoorientin, and vitexin were prepared in our laboratory [18,19]. Resins were purchased from Chemical Industry of Nankai University (Tianjing, China). Other chemicals and reagents were analytical grade from Beijing Chemical Works (Beijing, China). The reference compound, orientin, was accurately weighed and dissolved in a 60% aqueous ethanol solution (v/v) to prepare a standard stock solution. Working standard solutions for the calibration curves were prepared by diluting the standard solution with 60% aqueous ethanol solution (v/v) to the appropriate concentrations. All solvents and solutions were filtered through a nylon membrane (0.45 μm pore size) before HPLC.

3.2. Pretreatment of Adsorbents

Macroporous resins including D101, D4020, NKA-9, X-5 and AB-8 were soaked in 95% aqueous ethanol solution (v/v) for 24 h, loaded on chromatographic columns, first washed by 95% aqueous ethanol solution (v/v) until the eluate is no longer cloudy after adding water, and finally washed by distilled water thoroughly. Then the resins were soaked in 5% HCl for 4 h and washed by distilled water to neutral, soaked in 5% NaOH for 4 h and washed by distilled water to neutral, and finally dried at 70 °C in a drying oven. Prior to use in the adsorption experiments, the dried resins were immersed in ethanol for 12 h and subsequently the ethanol was replaced by distilled water through washing.

3.3. Preparation of the Crude Flavonoid Extracts from Scorzonera austriaca Herbs

According to the method reported in literature [18], 2 kg of air-dried whole S. austriaca herbs were extracted twice with 20 L of 70% aqueous ethanol solution (v/v) at room temperature. The extraction solution was concentrated under reduced pressure to remove ethanol, and the water concentrate was filtered and then passed through a D101 poly porous resin column eluting successively with water and 60% aqueous ethanol solution (v/v). The crude flavonoid extracts were obtained from 60% aqueous ethanol eluate by vacuum distillation recovery and used for the next experiments. The content of total flavonoids in the crude flavonoid extracts was 62.1% by using the following ultraviolet-visible spectrophotometry method and the content of orientin in the crude flavonoid extracts is 9.5% by using the following HPLC method.

3.4. Ultraviolet-Visible Spectrophotometry Method for Determination of Flavonoid Compounds

The concentration of total flavonoids was determined according to the following method. Certain amount of standard solution or sample solutions, 3.0 mL of 1% AlCl₃-MeOH and 3.0 mL of HAc-NaAc buffer solution (pH = 5) were transferred to a 10-mL volumetric flask and diluted with 60% aqueous ethanol solution (v/v) to volume, and after one hour of coloration the absorbance (A) was measured using a UV-VIS spectrophotometer at 390 nm in duplicate. A calibration curve, \( A = 19.676 X + 0.004 \) (\( R^2 = 0.9999 \), linear range: 0.001–0.0416 mg; mL⁻¹), was established by plotting the absorbance (A) of the reference compound, orientin, against the concentrations (X) of the standard solutions, and then the amounts of total flavonoids expressed as orientin equivalents in the samples were then calculated using the calibration curve.

3.5. HPLC/ESI/MS Analysis of Total Flavonoids

HPLC analysis of total flavonoids was carried out on a LC-10AT VP Plus system (Shimadzu, Kyoto, Japan). Analysis was performed on a Phenomenex Gemini ODS column (4.6 mm × 250 mm, 5 μm). The following gradient elution procedure was used for the analysis of the samples: The mobile phase components were acetonitrile (A) and 0.1% formic acid solution in water (B) and the linear gradient program was 3%–14% A from 0–7 min, 14%–17% A from 7–30 min, 17%–35 % A from 30–60 min, and 35%–100 % A from 60–65 min, the mobile phase flow rate was 0.8 mL·min⁻¹, the column temperature was kept at 25 °C, the injection volume was 10 μL, and the eluate was monitored.
at 337 or 207 nm. The mass spectra were obtained on a Bruker microOTOF-Q II mass spectrometer (Bruker Corporation, Bremen, Germany). Acquisition Parameters: Source Type: ESI; Focus: Active; Scan Begin: 50 m/z; Scan End: 3000 m/z; Ion Polarity: Positive; Capillary: 4500 V; End Plate Offset: −500 V; Collision Cell RF: 150.0 Vpp; Nebulizer: 0.8 Bar; Dry Heater: 180 °C; Dry Gas: 8.0 L min⁻¹; Divert Valve: Waste.

Identification of compounds was achieved by comparing their mass data with those reported in the literature and by using internal standard methods with available reference standards. The content of orientin was calculated with the regression equation, \( Y = 2.379601 \times 10^7 X - 69815.1 \) (\( Y \), peak area; \( X \), concentrations of the standard solutions; \( R^2 = 0.9998 \); linear range, 0.04–0.28 \( \mu g \cdot \mu L^{-1} \)) from the standard curve by using an external standard method. The results were averages of triplicate determinations.

3.6. Static Adsorption and Desorption Tests

The static adsorption and desorption tests of total flavonoids on macroporous resins were performed as follows: 1 g of pretreated resin together with 50 mL of 1.40 mg mL⁻¹ crude flavonoid extract solution was added into a flask, shaken (130 rpm) for 24 h at 25 °C. After adsorption, the resin was desorbed with 50 mL of 95% aqueous ethanol solution (v/v), and shaken (130 rpm) for 24 h at 25 °C. The respective concentrations of total flavonoids in the sample solution after adsorption of a certain time were monitored at equal time intervals till equilibration to get adsorption kinetic curves. The selectivity of resins was based on the capacities of adsorption and desorption, the ratio of desorption and the rate of adsorption. The tests for the maximum adsorption capacity on the selected resin were conducted by contacting 50 mL sample solutions at different concentrations with 1 g of pre-weighed resins. The effects of ethanol concentrations on the ratio of desorption were carried out by desorbing successively with aqueous ethanol solutions of different ethanol concentrations. All the processes were repeated for three times.

3.7. Dynamic Adsorption and Desorption Tests

Dynamic adsorption and desorption experiments for total flavonoids were carried out on glass columns (2.5 mm × 300 mm) wet-packed with 70 g (wet resin) of the selected hydrated resin. The bed volume (BV) of resin was 100 mL. After reaching adsorptive saturation, the column was first washed by distilled water with 5 BV, and then eluted by aqueous ethanol solutions. The gradient elution tests were taken as follows: the column was first washed by distilled water with 5 BV, and then eluted by different aqueous ethanol solutions, in which the eluate was collected every 20 mL and the elution process of each ethanol concentration was carried out until the eluate was colorless. The eluates were analyzed by TLC (plates: glass precoated silica gel GF₂₅₄ plates; developing solvent: CHCl₃-MeOH-EtOAc-H₂O, 2:2:4:1, v/v, lower layer; detection method: spraying with 10% H₂SO₄ in 95% ethanol followed by heating and leaving overnight), merged and then concentrated to dryness under vacuum. The effects of two variables, ethanol/water ratios and elute flow rates, on desorption properties of the selected resin were also studied. All the results were averages of triplicate determinations.

4. Conclusions

A method for the preparative separation and purification of total flavonoids from the crude flavonoid extracts of *S. austriaca* with macroporous resin has been successfully developed in this study. The static adsorption and desorption characteristics of five widely used macroporous resins for total flavonoids in the crude flavonoid extracts of *S. austriaca* were evaluated. Among the resins investigated, D4020 was selected as a suitable resin for total flavonoid separation because of its comparatively high adsorption capacity and ratio of desorption. Parameters of the initial concentration of the crude flavonoid extracts, elution solvent concentration and elute flow rate were optimized with the static/dynamic adsorption and desorption tests. The separation effects of the gradient elution had been also evaluated according to the color of the spots in TLC, and thus the optimal gradient
elution were selected. In conclusion, the appropriate preparation and purification process of total flavonoids from crude flavonoid extracts of *S. austriaca* on D4020 resin was as follows: the pretreated D4020 resin column was loaded with the crude flavonoid extract solution at the initial concentration of 20 mg·mL⁻¹, and after reaching adsorptive saturation, the column was eluted successively with 5 BV of water, 5 BV of 5% (v/v) aqueous ethanol and 5 BV of 30% (v/v) aqueous ethanol at the elute flow rate of 2 BV·h⁻¹. The total flavonoids were obtained from the eluate of 30% (v/v) aqueous ethanol by vacuum distillation recovery.

Using the D4020 resin at the optimal conditions, the total content of total flavonoids in the final product was 93.5%, which represents an improvement by about 150% after the preparative separation and purification procedure. The HPLC chromatograms recorded at the detection wave lengths of 337 and 207 nm demonstrated that this preparation and purification process can remove chlorogenic acid and terpenoids. Validation experiments showed that the method was stable and feasible.

This method has the advantages of low cost, high efficiency, procedural simplicity and ease in upscaling compared to the existing methods for total flavonoid purification such as high speed counter-current chromatography, solvent extraction, supercritical carbon dioxide extraction, aqueous two-phase flotation, and could be referenced for the preparative separation and purification of total flavonoids from *S. austriaca* in industry.

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**Author Contributions:** Guang-Shu Wang designed research, analyzed the data and wrote the paper; Yang Xie and Qiu-Shu Guo performed the research. All authors read and approved the final manuscript.

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Sample Availability: Samples of the compounds II–VI are available from the authors.