A NOVEL METHOD FOR EXTRACTION AND SEPARATION OF TOTAL FLAVONES AND TOTAL ASTRAGALOSIDES FROM *Radix astragali*

Zunfeng Liu, Xueping Liu, Dongping Liu, Jun Chen, Ruimin Sun, Ping Jiang, Zhirong Huang, and Fangxing Li*

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In this paper a novel method for extraction and separation of total flavones and total astragalosides from Radix astragali *using a resin method is suggested. Ethanol is used as the extractant, the macroporous absorption resin modeled H-107 is used to enrich the astragalus extract, and the macroporous strong base anion resin D-280 is used to separate the total flavones and the total astragalosides. This method allows a good yield of total flavones of 600 mg/100 g* Radix astragali, *a purity of 94.2%, and a yield of total astragalosides of 28 mg/100 g* Radix astragali.

Key words: *Radix astragali*, flavone, astragaloside, extract, separation.

As an important Chinese traditional medicine material, *Radix astragali* contains a high content of isoflavones, astragalosides, and polysaccharides [1]. Recent research has reported that astragalus isoflavones have the pharmacological activity of dilating coronary artery, increasing coronary vessel blood flow, and improving circulation in cardiac muscle capillaries; the astragalosides are used as a medicine for various ailments and diseases, such as blood pressure medicine, anti-inflammatories, or anti-depressants. [2, 3]. As far back as the 1990s various extraction methods for *Radix astragali*, such as water extracting [4], ethanol extracting [5], extraction refining [5, 6], resin absorbing [7, 8], and the supercritical-CO_2 method [4, 8] have been reported.

One of the present extracting methods for determining the contents of total flavones and total astragalosides in *Radix astragali* uses methanol as the extracting agent, a polyamine resin column to take the total flavones and total astragalosides up, water to wash the total astragalosides out, and finally methanol to wash the total flavones out [9]. As we know, astragalosides dissolves poorly in water, therefore, they can hardly be washed out of the polyamine resin using water as desorbing agent. On the contrary, they can be washed out with the flavones using methanol. Another method for extracting the *Radix astragali* uses different ethanol/water mixtures as the extracting agent, the astragalus extract is treated with the absorbing resin H-107, and then the resin is washed with 95% alcohol to get astragalosides containing spent regenerant; finally the spent regenerant is condensed and dried to get astragalosides [10]. Another method for extracting astragalosides from *Radix astragali* uses ethanol as the extracting agent treats the astragalus extract with the absorbing resin H-107, washes the resin with distilled water, and then washes the resin with ethanol to get astragalosides containing spent regenerant; finally the spent regenerant is condensed and dried to get the raw astragalosides [11]. In these two methods, the total flavones and total astragalosides can both be extracted from the *Radix astragali*, then absorbed by the resin H-107 and washed out of the resin without further separation. That is to say, the final product is a mixture of flavones and astragalosides, not the pure astragalosides.

The extracting method suggested in this paper uses ethanol to macerate the pulverized *Radix astragali* and then enriches the astragalus extract with an absorbing resin, finally separating the flavones and astragalosides using a strong base anion resin.

Astragalus polysaccharides are soluble in water but insoluble in ethanol; astragalosides are soluble in ethanol but slightly soluble in water; flavones are soluble in ethanol but insoluble in water. Therefore, we can divide these three components into two groups using an ethanol extractant or a water extractant: one is polysaccharides; the other is a mixture of astragalosides

Department of Chemistry, Nankai University, Tianjin 300071, P. R. China, e-mail: lifangxing@nankai.edu.cn. Published in Khimiya Prirodnykh Soedinenii, No. 1, pp. 26-29, January-February, 2007. Original article submitted November 22, 2005.

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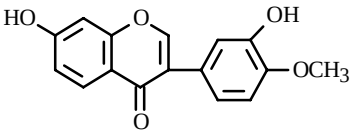
and flavones. The content of astragalosides and flavones in *Radix astragali* is very small, only 0.8 mg/g *Radix astragali* and 0.4 mg/g *Radix astragali*, respectively. If we use water as the extractant, a small part of the astragalosides and flavones will be extracted with the polysaccharides. Therefore, we macerate the *Radix astragali* to get the astragalosides and flavones using ethanol first, and then the leaves are used to extract polysaccharides.

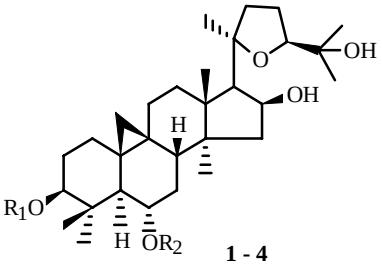
There are astragalosides, flavones, a small amount of polysaccharides and amylum, and a large amount of sugar in astragalus extract. The polysaccharides and amylum may settle and are then removed when the astragalus extract is cooled to room temperature. The sugar should also be removed because it can be absorbed by the absorbing resin at the subsequent enrichment step and then decrease the absorbing efficiency of flavones into the absorbing resin. When the astragalus extract is kept for 72 h or more, the majority of the sugar will separate out, leaving a small amount of sugar in the astragalus extract, which can be removed in the enrichment step.

Because the content of flavones and astragalosides in astragalus extract is very small, an enrichment step is needed. Pass the astragalus extract through the absorbing resin column; the astragalosides, flavones, and the sugar will be absorbed onto the resin. If we wash the resin with a solvent having a good selectivity for astragalosides and flavones, they will be enriched, and the remaining sugar can be removed.

After the enrichment step, we get a concentrated solution of flavones and astragalosides, which needs further separation. Note that there are some phenolic hydroxyl groups in flavone C, which can be loaded into a strong base anion resin, while astragalosides **1–4** do not have exchangeable groups with the strong base anion resin [12].



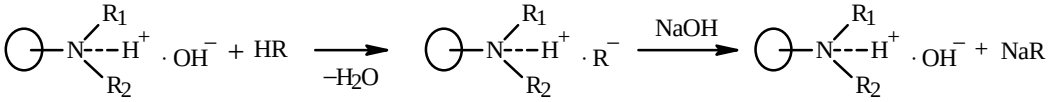
Flavone C



1 - 4

1: R_1 = 2,3-*O*-Diacetyl-*β*-D-Xyl*p*, R_2 = *β*-D-Xyl*p*
2: R_1 = 2-*O*-Acetyl-*β*-D-Xyl*p*, R_2 = *β*-D-Xyl*p*
3: $R_1 = R_2$ = 2,3,4-Triacetyl-*β*-D-Xyl*p*
4: R_1 = 2,3-*O*-Diacetyl-*β*-D-Xyl*p*, R_2 = D-Xyl*p*

The reaction of flavones with the strong base anion exchange resin is shown in Scheme 1.



Scheme 1. The reaction between flavones and the strong base anion resin HR: schematic representation of the flavone molecule.

Seventy grams of pulverized *Radix astragali* and 250 mL of ethanol were added into a 500 mL four-neck flask and then warmed to 78°C under stirring and maintained for different times.

The extracting efficiency increases with increasing extracting time (**1**) and almost remains constant when the extracting time (**1**) is longer than 2 h (**2**).

The absorbing resin H-107 is employed in this paper because of its very good absorbing ability [13]. Pass 600 mL of astragalus extract through the absorbing resin column at different rates (1 mL/min and 3 mL/min). The content of flavones in the effusion is determined every 30 mL effusion. The experimental results are shown in Fig. 1 (*1*, *2*).

It can be seen from the Fig.1 that a small leak was observed at the fifth bedvolume on curves *1,3* (at a flow rate of 3 mL/min), at the eighth bedvolume on curve *2*, and fifteenth bedvolume on curve 4. No leak is observed for column 5. The leak velocity of curve *2* is much slower than that of 1 due to better absorbance of flavones onto the resin.

TABLE 1. Concentrations of General Astragalosides in Spent Regenerant at Different Velocities of Flow

mL/min	BV (1) (mg/mL)	BV (2) (mg/mL)	BV (3) (mg/mL)	BV (4) (mg/mL)
0.2	0.451	0.446	0.445	0.440
0.4	0.462	0.458	0.452	0.449

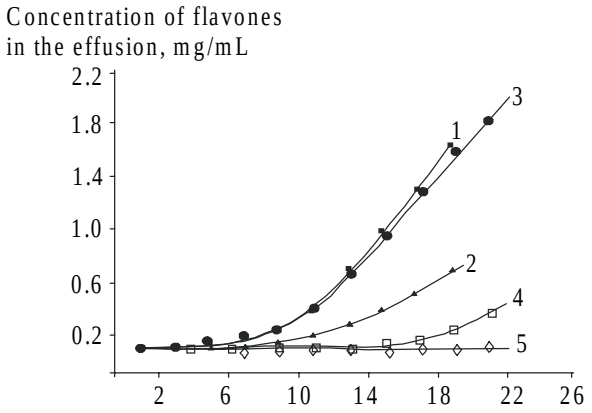


Fig. 1

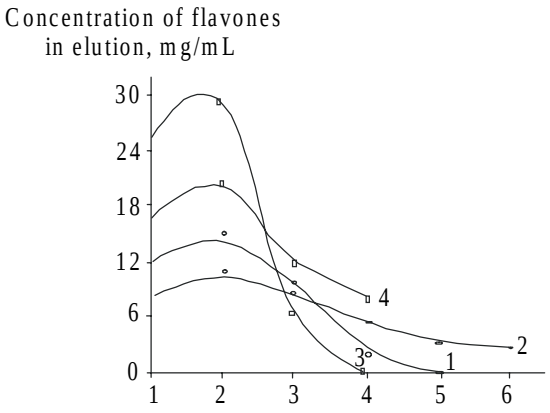


Fig. 2

Fig. 1. Loading curves of flavones on the absorbing resin at different flow rates (1 – 3 mL/min; 2 – 1 mL/min) and on multiple absorbing resin columns (3 – column a; 4 – column b; 5 – column c).

Fig. 2. Elution curves of flavones and astragalosides with hot ethanol of 50°C at different flow rates (elution BV (30 mL): 1 - 0.2 mL/min; 2 - 0.4 mL/min); (elution BV (15 mL):3 - 0.2 mL/min; 4 - 0.4 mL/min).

In order to avoid the loss of flavones and astragalosides, the absorbing efficiency of the multi-column absorbing resin is tested: 800 mL of 4.28 mg/mL of astragalus extract is passed through 30 mL absorbing resin column (**3**); we collect the effusion from the fourth bedvolume and pass it through 30 mL of absorbing resin column (**4**), then collect the effusion from the fourth bedvolume and pass it through 30 mL of absorbing resin column (**5**).

It is considered that the amount of molecules taken onto the absorbing resin influences the equilibrium of flavones and astragalosides between the solid-liquid phases. The thinner the solution, the less the pore of the absorbing resin will be taken up when the solution flows through, and the better the absorbing efficiency. Therefore, multiple-column absorbing is much better than single-column absorbing. In this paper, the optimum absorbing condition is three-column absorbing.

Flavones and astragalosides are very soluble in hot ethanol; therefore, hot ethanol of 50°C is used as the washing agent. Hot ethanol of 50°C is flowed through the above absorbing resin column at different flow rates (0.2 mL/min and 0.4 mL/min) to yield a carmine effusion. The absorbance is determined every 15 mL effusion. The elution curves at different flow rates are shown in Fig. 2.

Figure 2 shows that the concentration increases from 12.03 (25.36) mg/mL to 15.21 (29.78) mg/mL for curves 1 and 3 and from 8.21 (16.78) mg/mL to 10.97 (20.34) mg/mL for curves 2 and 4 respectively. Then curves 1 and 3 decrease sharply and reaches 0 mg/mL at the fifth bedvolume (curve 1) and at the fourth bedvolume (curve 3). Curve 2 is still high at the fifth bedvolume and curve 4 is still 8.35 mg/mL at the fourth bedvolume. Consequently, the optimum flow rate of hot ethanol is 0.2 mg/mL.

Pass 60 mL of carmine spent regenerant having a concentration of 12.01 mg/mL which flowed out from the absorbing resin through a 30 mL strong base anion exchange resin (D280) column at different flow rates (0.2 mL/min and 0.4 mL/min). We get a colorless solution. The disappearance of the carmine illustrates that the flavones are completely loaded into the strong base anion resin column. Condense and dry the effusion to get a powder, which represent the total astragalosides. After washing the total astragalosides with a small amount of water, dry it in air at room temperature. Weigh the powder and calculate the concentration of astragalosides in the effusion. The experimental results are listed in Table 1.

It can be seen from Table 1 that the content of astragalosides in the spent regenerant is almost the same at 0.2 mL/min and 0.4 mL/min. Therefore, the optimum flow rate is 0.4 mL/min.

Flavone A 5% NaOH 1:1 (v/v) ethanol–water solution was passed through the loaded strong base anion exchange resin column at different flow rates (0.2 mL/min and 0.4 mL/min) to elute the flavones. Collect the elution every 15 mL as a bedvolume and calculate the concentration of flavones; the experimental results are shown in Fig. 2.

In conclusion, we developed a novel method to isolate large amounts of total flavones and total astragalosides from *Radix astragali* root. Given the biological properties of astragalus isoflavones of dilating the coronary artery, increasing coronary vessel blood flow, and improving circulation in cardiac muscle capillaries, astragalosides can be used as a blood pressure medicine and as anti-inflammatories or anti-depressants; the method here can be scaled-up for industrial applications. This could provide a low-cost means to use a currently Chinese traditional medical material to develop commercial products, including new nutritional products with potential health benefits.

EXPERIMENTAL

Materials and Chemicals. *Radix astragali* (kindly supplied by Inner Mongolia, Baotou, China), absorbing resin H-107 (Cross-linked polystyrene, pearl size: 0.3–1.25 mm, purchased from the chemical plant of Nankai University, Tianjin, P.R.C) and macroporous strong base anion resin D-280 (Styrene-DVB, functionality: $-N^{+}(CH_3)_3$, pearl size: 0.32–1.25 mm, purchased from the chemical plant of Nankai University, Tianjin, P.R.C), ethanol (95%, AR).

Maceration Extraction of Flavones and Astragalosides. Put 70 g of pulverized *Radix astragali* in a flask, and then add 250 mL ethanol to macerate the *Radix astragali* for a period of time at a certain temperature. Then filter the astragalus extract to remove the suspensoid. Let the filtrate stand for at least 72 h, there will be some sugar separating out, which can be identified using IR analysis.

Enrichment of Astragalus Extract. Pass the pretreated astragalus extract through 30 mL of pretreated absorbing resin column H-107; the remaining ethanol is removed from the resin by vacuum aspiration and the absorbed flavones and astragalosides are eluted with hot ethanol at 50°C.

Separation of Flavones and Astragalosides. Pass the spent regenerant through 30 mL of macroporous strong base anion resin (D280) column, and then concentrate and dry the effusion to get the raw total astragalosides. Then wash the resin with hot ethanol at 50°C to get the total flavones.

Evaluation Index of Content of the Astragalosides and Flavones in Solution. Flavones contain π electrons and strongly absorb in the ultraviolet. Therefore, the content of flavones in solution can be determined by UV analysis. Astragalosides do not absorb in the ultraviolet. Astragalosides exist in solution or on the resin with the flavones before the final separation step. Consequently, we can evaluate the contents of astragalosides and flavones in solution by only determining the content of flavones.

Preparation of the Standard Sample. The standard sample in this paper is homemade. After the final separation step, the raw flavones are washed with a small amount of water and then recrystallized from ethanol. Repeat the operation three times, and the refined product is used as the standard sample. The stock solution of flavones of 0.50 mg/mL is prepared in ethanol. A 1 mL aliquot of the stock solution is transferred into a 10 mL volumetric flask and then diluted to the mark with ethanol to obtain the standard mixture solution. This mixed standard solution is then diluted step by step to obtain a series of working solutions. All stocking solutions and working solutions are stored at room temperature. Then the absorbance is measured in the endpoint mode at 280 nm on a UV/VIS microplate spectrophotometer (WFZ800-D3A). The calibration curve is $y = 0.06x$.

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