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Optimizations of Conditions for Maximum Recovery of Astragalin from *Thesium chinense* Turcz

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Abstract: Astragalin, one of the flavonoid extracted from plants is increasingly in demand in food and pharmaceutical industries due to its various biological and physiological activities including antioxidation, antiallergy, anti-inflammation and anti-hepatoptoxic. The whole plant of *Thesium chinense* Turcz is a major source of astragalin. This study developed a low-cost process encompassing the efficient extraction, fractionation, isolation and recrystallization to obtain high-purity astragalin from *Thesium chinense* and it could improve the economic utilization of this plant. In this study plant was extracted with 80% ethanol for 3 h and extract was fractionated on AB-8 (styrene based copolymer) with water, 20, 40 and 80% aqueous ethanol. Astragalin was isolated by silica gel column and purified by recrystalization with methanol. These conditions resulted 90% recovery of Astragalin with 95% purity.

Key words: Astragalin, *Thesium chinense* Turcz, extraction, fractionation, recrystallization, antioxidation

INTRODUCTION

Astragalin (kaempferol-3-O-Gluoside; Fig. 1) is one of the major flavonoid found in a variety of plants. Astragalin is receiving increasing attention due to its various health benefiting and biological activities, including antioxidative (Parejo *et al*., 2004; Correia *et al*., 2006), anti-inflammatory (Kovganko *et al*., 2004; Correia *et al*., 2006), Anti- HIV (Schinazi *et al*., 1997), anti-allergic effects (Matsumoto *et al*., 2002). Besides this astragalin is responsible for the color of different beans and has the potential to extract it and market as nutritionally important food supplement (Beninger and Hosfield, 1999).

Thesium chinense Turcz. belongs to family Santalaceae. It is grown as annual herb throughout the China. The whole plant is claimed to possess medicinal properties (Anonymous, 2003). In traditional Chinese medicines it is used for inflammation, sore throat, pneumonia, cough and headache etc. Phytochemical investigation reveals the presence of flavonoids, glycosides, essential oils, alkaloids, steroids and organic acids (Lu and Wang, 2004). They also reported the isolation of five flavonoids including astragalin from this plant. However, low-cost downstream processes for recovering this major flavonoid from this plant, have not been studied yet.

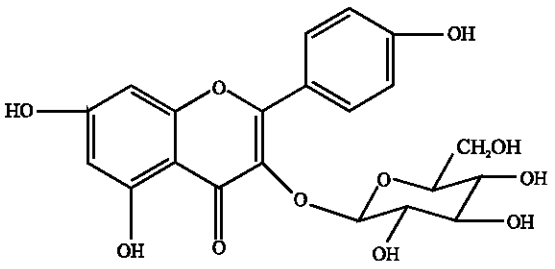

Fig. 1: Chemical structure of Astragalin (kaempferol-3-O-glucoside)

AB-8 (cross-linked polystyrene copolymer) is a durable slightly hydrophilic polymer having high adsorption capacity with possible recovery of the adsorbed molecules, relative low cost and easy regeneration. They are currently used for adsorption of flavonoids and other components extracted from many plants (Mauro *et al*., 1999, 2000; Xueming, 2004). In this study we used this resin for fractionation of extract.

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The aim of this study was to develop an effective, low cost and industrially feasible method for extraction, fractionation and crystallization of Astragalin from *Thesium chinense*.

MATERIALS AND METHODS

General experimental procedure: Fractionation was performed over AB-8 resin (chemical industrial company affiliated to Nan Kai University, Tianjin, China). Silica gel for column chromatography (Ocean chemical industry, China). HPLC was carried out on Elite HPLC (Dalian scientific instrument Co., Limited). All chemicals used in study were of analytical grade.

Plant material: The whole plats of *Thesium chinense* Turcz (Santalaceae) were collected from Anhui province of China and identified by botanist. A voucher specimen has been deposited in the Beijing Institute of Technology, Beijing, China.

Extraction of Astragalin:

Extraction of Astragalin from *Thesium chinense* Turcz: One hundred gram of completely air-dried whole plant of *Thesium chinense* (comprising leaves, flowers, stalks, roots and seeds) were ground and sieved to obtain the particle size of less than 2 mm. The ground material was extracted with 1 L of one of the following aqueous (aq.) solvents: water, methanol (80%, v/v), ethanol (80%, v/v) and acetone (80%, v/v) under reflux. The extraction temperature and time were chosen in the ranges of 30-100°C and 1-4 h, respectively. After the extraction, the extracted slurry was filtered to collect the extract alone. The above procedures were repeated thrice and the extracts obtained from the first, second and third extractions were combined and concentrated at 50°C using a vacuum evaporator. Dry residue was dissolved in water for further fractionation

Fractionation of extract: Fractionation of the water extract containing Astragalin was carried out by AB-8 resin (cross-linked polystyrene copolymer)

Preparation of AB-8 Column: AB-8 resin was packed into column, washed with ethanol to remove the impurities and then with water to dispel the ethanol. Extract was poured into the column and washed with water to remove sugar, acids and other water-soluble molecules. Then column was washed with 20, 40 and 80% ethanol (v/v) .The eluted fractions were concentrated to dryness at 50°C under a reduced pressure.

Isolation of Astragalin: Astragalin was isolated from 40% ethanol extract by silica gel column using chloroform and methanol (8:2). Crude Astragalin was obtained which was purified by recrystallization.

Recrystallization of Astragalin: Using methanol did the recrystallization of astragalin. Crystals were filtered off and air-dried. The final purity of the obtained Astragalin was determined by high-performance liquid chromatography by following a typical HPLC protocol for analyzing flavonoid glycosides (Simony *et al*., 2005). Measurements of astragalin concentrations by HPLC were repeated at least two times and the standard errors for the replicated samples were typically within 3%. These conditions resulted 90% recovery of Astragalin with 95% purity.

Statistical analysis: Means and standard errors of experimental data was calculated.

RESULTS AND DISCUSSION

Effect of extraction solvents: To find an effective solvent for the extraction of Astragalin from *Thesium chinense,* various solvents were tested as shown in Fig. 2. The use of aqueous ethanol and methanol (both 80%, v/v) as extraction solvents produced the highest yields of astragalin: 736 and 736.5 mg of Astragalin from 100 g of dry plant material, respectively. In our subsequent extraction experiments, 80% ethanol was used due to the lower toxicity of ethanol compared to the other solvents tested in this study (e.g., acetone and methanol).

Effect of extraction temperature: To establish an optimal extraction temperature, the extraction was performed with 80% (v/v) ethanol at temperatures of 30-100°C as shown in Fig. 3. The extraction yield of Astragalin increased with temperature until reaching its maximum (736.5 mg of

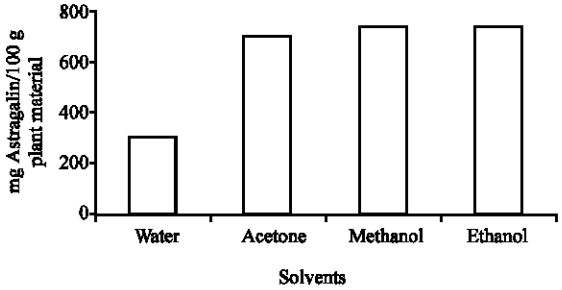

Fig. 2: Effect of aqueous extraction solvents (80% v/v) and water alone on the recovery yield of Astragalin from *Thesium chinense* Turcz

Table 1: Fractionation of Astragalin on a styrene-based column with water and aqueous ethanol

	Total dry solids		Astragalin	
Solvents	Weight (g) (% w/w)	Recovery yield (% w/w)	Purity (%)	Recovery yield (% w/w)
Water	2.1±0.1	37.8±0.5	0.0±0.0	0.0±0.00
20% ethanol	0.9±0.3	18.8 ± 0.3	2.3±0.0	0.5±0.01
40% ethanol	2.0±0.1	37.7±0.1	70.0±0.5	95.0±0.05
80% ethanol	0.3±0.1	5.6±0.2	2.1±0.01	0.8±0.02

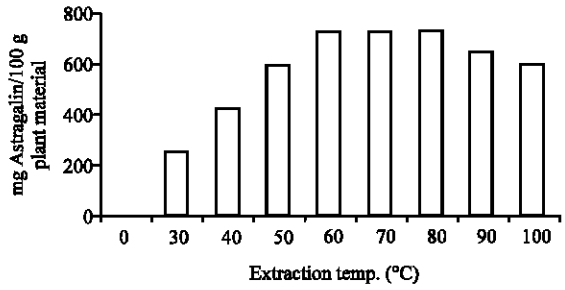

Fig. 3: Effect of extraction temperature on the recovery yield of Astragalin from *Thesium chinense* Turcz with (80% v/v) aqueous ethanol

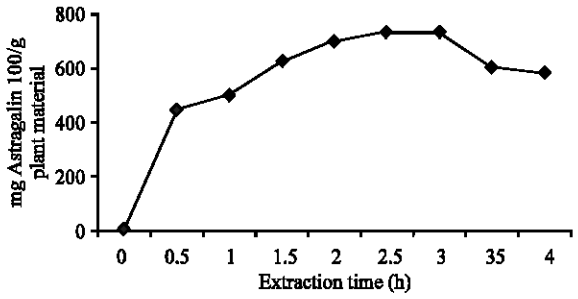

Fig. 4: Effect of extraction time on the recovery yield of Astragalin from *Thesium chinense* Turcz with (80% v/v) aqueous ethanol at 80°C

astragalin from 100 g dry weight) at 80°C and subsequently decreased at 100°C. Since at higher temperatures increase in the extraction yield was very large from 30-60°C, extraction temperatures in the range 60-80°C were considered optimal for achieving a high recovery yield of Astragalin.

Effect of extraction time: In this experiment, under optimal conditions determined from previous sections (80% ethanol at 80°C), the extraction was performed for varying times to find optimal extraction time. As demonstrated in Fig. 4 extending the extraction time from 1-4 h ,the yield was maximum at 3 h further increases in the extraction time resulting in a slight decrease in the yield; this was probably due to the loss of Astragalin at the high extraction temperature used (80°C). Therefore, the optimal extraction time was considered to be 3 h.

Fractionation of extract using a polymeric adsorbent: Astragalin was extracted from *Thesium chinense* plant under the optimal conditions determined in the previous Sections (80% aqueous ethanol and 80°C for 3 h) and the extract solution was concentrated and dissolved in water. After loading the water extract onto a styrene-based adsorption resin column, the column was washed with water, 20, 40 and 80% (v/v) aqueous ethanol. The results in Table 1 showed that water and 20% aqueous ethanol preferentially dissolved the highest proportion of more hydrophilic fraction relative to Astragalin: eluting with 40% aqueous ethanol yielded 90% (w/w) of total astragalin while the elution with water, 20 and 80% aqueous ethanol gave only 5% of total Astragalin in *Thesium chinense*.

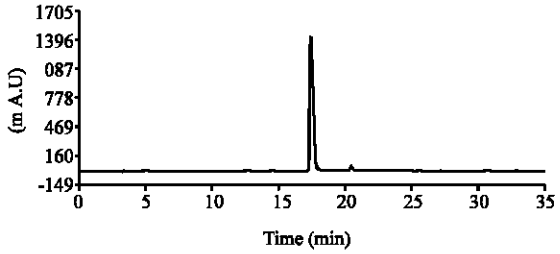

Fig. 5: HPLC chromatogram of Astragalin purified from the extract of *Thesium chinense* Turcz

Recrystallization of Astragalin: Crude astragalin obtained from silica gel column was almost 80% pure. The recrystallization of crude Astragalin was done with methanol. Recrystallized Astragalin, which was almost insoluble in methanol removed by filtration and air-dried. HPLC analysis (Fig. 5) revealed that the purity of the Astragalin crystals was 95%. with the final recovery yield of astragalin for the overall separation and purification processes involving extraction fractionation and recrystallization steps being as high as 90%.

CONCLUSIONS

This study determined the optimal extraction, fractionation and recrystallization conditions for recovering astragalin with high purity under optimal conditions established, 90% of total astragalin was

obtained with 95% purity through relatively low cost process. This economically feasible process can be readily applied in pharmaceutical industries demanding high purity astragalin with high value and can also promote the economic utilization of *Thesium chinense*.

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