



Analytical Methods

Optimisation of the microwave-assisted extraction process for four main astragalosides in Radix Astragali

Ming-Ming Yan¹, Wei Liu¹, Yu-Jie Fu^{*}, Yuan-Gang Zu^{*}, Cai-Yun Chen, Meng Luo

Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China

Engineering Research Center of Forest Bio-preparation, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China

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ABSTRACT

An efficient microwave-assisted extraction (MAE) technique was employed in extracting astragalosides I–IV from Radix Astragali. Astragalosides were quantified by liquid chromatography–electrospray ionisation mass spectrometry (LC-ESI/MS). The MAE procedure was optimised, validated and compared with other conventional extraction techniques. MAE gave the best result due to the highest extraction efficiency within the shortest extraction time. The optimal conditions of MAE were: employing 80% ethanol as solvent, ratio of solid/liquid 1:25 (g/ml), temperature 70 °C, irradiation power 700 W and three extraction cycles, each 5 min. Scanning electron microscopy (SEM) images of Radix Astragali materials after different extractions were obtained to provide visual evidence of the disruption effect. This is the first report on combining MAE with LC-ESI/MS for the extraction and quantification of astragalosides I–IV in Radix Astragali. The developed MAE method provided a good alternative for the extraction of triterpenoid saponins in Radix Astragali as well as other herbs.

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1. Introduction

Radix Astragali (Huangqi in Chinese), the dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge, is one of the most famous and frequently used herbal medicines and food additives. Nowadays, various Radix Astragali preparations are commercially available, and Radix Astragali products are widely used not only as efficacious medicinal prescriptions, but also as healthy food supplements (Qi et al., 2008). It has been used for over 2000 years in traditional Chinese medicine (TCM) prescriptions for the treatment of nephritis, diabetes, albuminuria, hypertension, cirrhosis, cancer, as well as a sedative and tonic (Li et al., 2001; Wang, Li, Yu, Zhao, & Ding, 2004). It could also be used in stewing fish, stewing chicken and making tea as a flavouring agent and additives of food in China. Chemical constituent investigations on Radix Astragali resulted in the discovery of several kinds of bioactive compounds like triterpene saponins, isoflavonoids, polysaccharides, amino butyric acids and various trace elements (Lin et al., 2000; Qi et al., 2006). In gen-

eral, triterpenoid saponins represent the major beneficial compounds responsible for the bioactivities and efficacies of Radix Astragali on human health (Qi et al., 2009). Especially, the four astragalosides studied in the current investigation, i.e., astragaloside I (AG I), astragaloside II (AG II), astragaloside III (AG III) and astragaloside IV (AG IV), belong to cycloartane-type triterpenoid saponins. They are considered as the major bioactive constituents with better pharmacological activities and possess antioxidant (Luo et al., 2004; Zhang et al., 2007), antitumour (Cho & Leung, 2007), hepatoprotective (Gui et al., 2006), anti-diabetic, antimicrobial, antiviral and immunological activities (Song, Kobayashi, Xiu, Hong, & Cyong, 2000). Their chemical structures are shown in Fig. 1. Thereinto, AG III and AG IV represent a pair of isomeric compounds (Zu et al., 2009).

The conventional liquid–solid extraction techniques, such as Soxhlet extraction (SE), heat reflux extraction (HRE), ultrasonic extraction (UE) and maceration extraction (ME) are discommodious, laborious, time-consuming and require large volumes of toxic organic solvents, so increasing attention is paid to the development of more efficient extraction methods for the rapid extraction of active compounds from materials. Accordingly, fast, solvent-free and low-cost extraction techniques for the extraction of astragalosides from Radix Astragali are desirable. Microwave-assisted extraction (MAE) has been accepted as a potential and powerful alternative to conventional extraction techniques in the extraction of organic compounds from plant materials. The mechanical effects of the internal heating based on conduction and dielectric polarisation

^{*} Corresponding authors. Address: Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China (Y.-J. Fu). Tel./fax: +86 451 82190535 (Y.-J. Fu), tel.: +86 451 82191517; fax: +86 451 82102082 (Y.-G. Zu).

E-mail addresses: yujie_fu2002@yahoo.com (Y.-J. Fu), zygorl@vip.hl.cn (Y.-G. Zu).

¹ These authors contributed equally to this work.

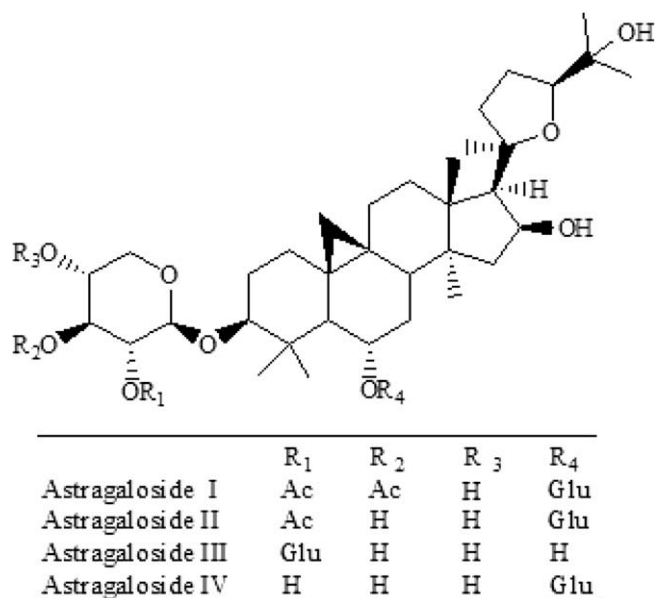


Fig. 1. Chemical structures of four astragalosides in Radix Astragali. Glu: glucose.

caused by microwave irradiation, and pressure builds up within the cells of the sample leading to an efficient delivery to plant materials through molecular interaction with the electromagnetic field and a rapid transfer of energy to the extraction solvent and raw plant materials (Eskilsson & Bjorklund, 2000; Rostagno, Palma, & Barroso, 2007). The MAE process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of ions, which enhance the penetration of solvent into matrix and release the intracellular product by disrupting the cell wall, allowing the dissolution of components to be extracted (Hemwimon, Pavasant, & Shotipruk, 2007). The MAE technique possesses many advantages compared to other methods for the extraction of compounds such as saving in processing time and solvent consumption (Chee, Wong, & Lee, 1996; Pastor, Vázquez, Ciscar, & Guardia, 1997; Xiong et al., 1999), and gaining high extraction efficiency.

In the present study, the objective was to evaluate the performance of MAE for four astragalosides in Radix Astragali and to optimise the MAE operating parameters. The parameters, which might affect the extraction efficiency, including ethanol concentration, ratio of solid/liquid, irradiation time, extraction temperature, microwave irradiation power and extraction cycles were studied in details. Astragalosides in Radix Astragali were directly quantified by LC-ESI/MS. To the best of our knowledge, this is the first report on combining MAE with LC-ESI/MS for astragalosides I–IV in Radix Astragali. The extraction efficiency of MAE was compared with conventional extraction techniques based on studying the extraction kinetics. The structural disruption to Radix Astragali with different extraction methods was observed by scanning electron microscopy (SEM).

2. Materials and methods

2.1. Chemicals and reagents

Astragalosides I and II ($\geq 98.0\%$) were obtained from ChromaDex (Santa Ana, USA), Astragaloside III ($\geq 95.0\%$) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Astragaloside IV and Ginsenoside Rg1 ($\geq 95.0\%$) were bought from Fluka (Switzerland) and Ginsenoside

Rg1 was used as the internal standard (IS). Acetonitrile and methanol were chromatographic grade (J&K Chemical Ltd., China). Formic acid was chromatographic grade (Dima Technology Inc., USA). Ethanol was analytical grade (Tianjin Chemical Reagents Co., Tianjin, China). Deionised water was purified by a Milli-Q Water Purification system (Millipore, MA, USA). All solutions and samples prepared for chromatographic analysis were filtered through $0.45 \mu\text{m}$ nylon membranes before injecting into LC-ESI/MS.

2.2. LC-ESI/MS conditions

The LC system consisted of an Agilent 1100 series HPLC system equipped with G1312A Binpump and G1379A Degasser (Agilent, San Jose, CA, USA). Chromatographic separation was performed on an Agilent Eclipse XDB-C18 column ($150 \text{ mm} \times 4.6 \text{ mm i.d.}$, $5 \mu\text{m}$). The mobile phase consisted of 0.05% formic acid aqueous solution (A) and acetonitrile (B). The following gradient elution program was used for separation: 0–6 min, 66% (A); 6–8 min, $66\text{--}55\%$ (A); 8–13 min, 55% (A); 13–14 min, $55\text{--}66\%$ (A); 14–17 min, 66% (A). The flow rate was 0.7 ml/min , the injection volume was $10 \mu\text{l}$. After 17 min of re-equilibration, the column was ready for a new injection. The column temperature was maintained at $30 \text{ }^\circ\text{C}$. Using this LC conditions, the chromatograms showed well-separated resolution, satisfactory peak shape as well as relatively short analysis time. A representative separation of astragalosides was achieved within 17 min (see Fig. 2).

The quantifications of astragalosides I–IV were performed by the internal standard method. The calibration curves of AG I, AG II, AG III and AG IV showed good linearity over the ranges of $0.054\text{--}27.0$, $0.046\text{--}23.0$, $0.032\text{--}16.0$ and $0.038\text{--}19.0 \mu\text{g/ml}$, respectively. The regression equations were $Y = 0.0176X + 0.0178$ ($R^2 = 0.9959$, $n = 8$), $Y = 0.0250X + 0.2515$ ($R^2 = 0.9912$, $n = 8$), $Y = 0.0431X + 0.0474$ ($R^2 = 0.9961$, $n = 8$) and $Y = 0.0366X + 0.0017$ ($R^2 = 0.9922$, $n = 8$), where Y is the peak area ratio of analyte to IS, X is the concentration of analyte ($\mu\text{g/ml}$). The LODs ($S/N = 3$) and LOQs ($S/N = 10$) for the analytes were less than 6 and 15 ng, respectively.

An API3000 Triple tandem quadrupole mass spectrometry with a Turbolon-Spray interface from Applied Biosystems (USA) was operated in positive electrospray ionisation (ESI⁺) source mode. All mass spectra were acquired in multiple reaction monitoring (MRM) transitions. The analytical conditions were as follows: the ion source was operated at a temperature of $250 \text{ }^\circ\text{C}$. The nebulising gas (NEB), curtain gas (CUR) and collision gas (COL) were set at 12, 10 and 6 a.u., respectively. The ion spray voltage (IS) was 5500 V. The entrance potential (EP) and focusing potential (FP) were set at 10 and 400 V. The declustering potential (DP) were 75 V for AG I, 60 V for AG II, 45 V for AG III, 120 V for AG IV and 25 V for IS; The collision energy (CE) were 32 V for AG I, 41 V for AG II, 27 V for AG III, 15 V for AG IV and IS; The collision cell exit potential (CCEP) were 7 V for AG I, 9 V for AG II, 6 V for AG III, 8 V for AG IV and 6 V for IS. The peak area corresponding to the transitions were m/z 869.9 \rightarrow 143.1 for AG I, m/z 827.7 \rightarrow 143.1 for AG II, m/z 785.8 \rightarrow 143.1 for AG III and AG IV, and m/z 801.7 \rightarrow 621.6 for IS. The MS parameters were manually tuned to obtain the highest response for each of the precursor/product ion combinations. Analyst software (version 1.4) installed on a DELL computer was used for data acquisition and processing.

2.3. Extraction procedures

A. membranaceus (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao was purchased from local Chinese medicinal materials market in Harbin, China and authenticated by Prof. Shao-Quan Nie from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, PR China. Voucher specimens were

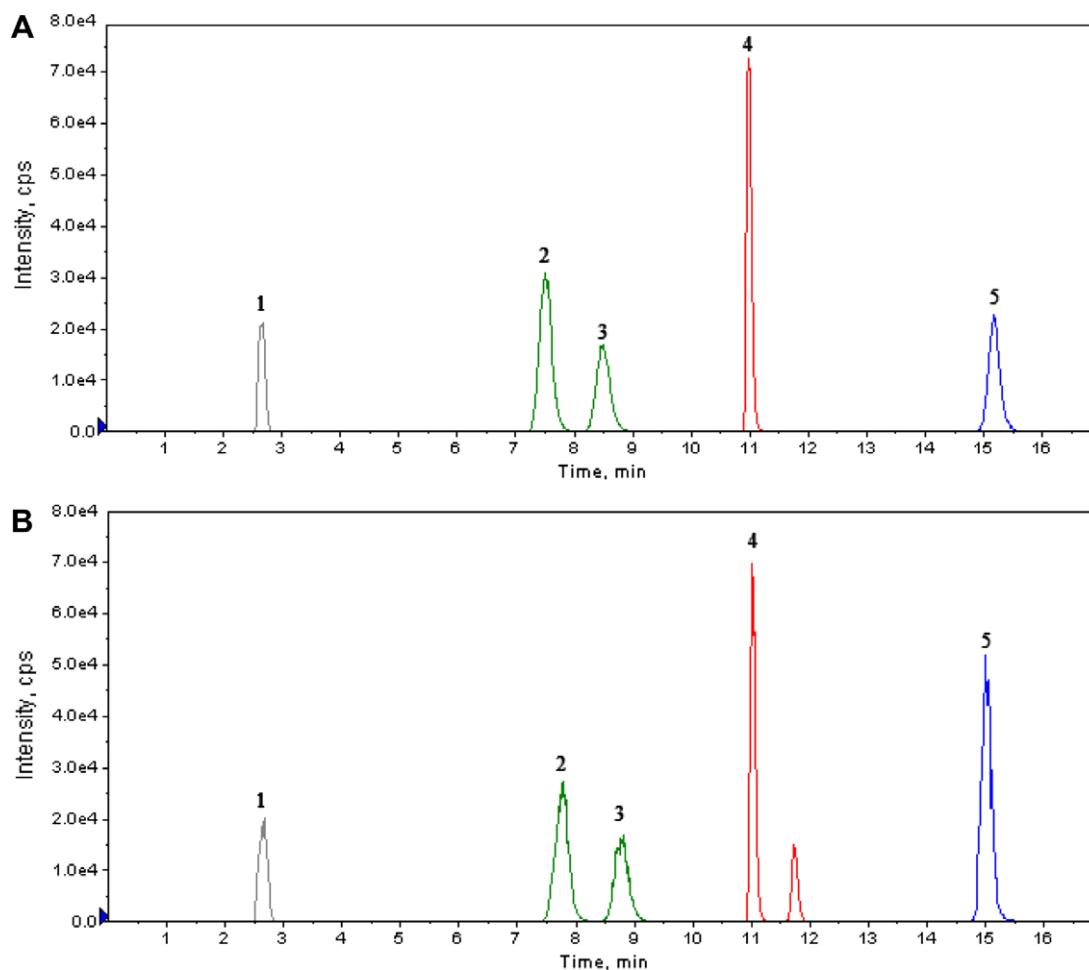


Fig. 2. Representative LC-ESI/MS chromatograms of (A) standard mixture and (B) sample of Radix Astragali with MRM. 1, IS; 2, AG IV; 3, AG III; 4, AG II; 5, AG I.

deposited in the herbarium of this Key Laboratory. Dried roots were powdered into a homogeneous size by a disintegrator (HX-200A, Yongkang Hardware and Medical Instrument Plant, China), and then sieved (20–40 mesh). The materials (5.0 g) were extracted with different techniques (microwave-assisted extraction, Soxhlet extraction, heat reflux extraction, ultrasonic extraction and maceration extraction) individually for obtaining the optimised extraction procedure.

2.3.1. Microwave-assisted extraction (MAE)

MAE was carried out in a MARS-II (1000 W, 2450 MHz) microwave accelerated reaction system from SINEO Microwave Chemistry Technology (Shanghai, China). The materials (5.0 g) and IS (1.0 mg) were accurately weighed, mixed and introduced into a special three-neck reaction flask. According to the experimental design, solvent was added into the flask and the flask was then connected to cooling water and a temperature sensor was inserted to measure and control the internal temperature after being placed in the microwave resonance cavity. The extraction process was performed under different conditions. The effects of ethanol concentration, ratio of solid/liquid, irradiation time, extraction temperature, irradiation power and extraction cycles on the extraction yields of four astragalosides were investigated.

2.3.2. Soxhlet extraction (SE)

The materials (5.0 g) and IS (1.0 mg) were mixed and placed in a Soxhlet apparatus and extracted with 100 ml of ethanol–water (80:20, v/v) solution for 4 h under a temperature of 90 °C.

2.3.3. Heat reflux extraction (HRE)

The materials (5.0 g) and IS (1.0 mg) were weighed, mixed and added into a round-bottom flask with 100 ml of ethanol–water (80:20, v/v) solution, the flask was placed into a water bath and connected with cooling water, and then allowed to reflux for 1 h under a temperature of 90 °C, this process was repeated three cycles.

2.3.4. Ultrasonic extraction (UE)

The materials (5.0 g) and IS (1.0 mg) were mixed and put into a conical flask and 100 ml of ethanol–water (80:20, v/v) solution was added to it, and this was then extracted in an ultrasonic bath (Kunshan Ultrasonic Instrument Co. Ltd., China) for 40 min with the power of 100 W, this extraction process was repeated three cycles.

2.3.5. Maceration extraction (ME)

The materials (5.0 g) and IS (1.0 mg) were accurately weighed, mixed and added to a 250 ml flask with 100 ml of ethanol–water (80:20, v/v) solution and macerated for 12 h.

After the extraction step, the filtered solutions were concentrated to dryness in a rotary evaporator device (RE-52AA, Shanghai Huxi Instrument Co., China) under vacuum at 55 °C. The obtained dry extracts were diluted in methanol (2 ml, HPLC-grade). The sample was further 500-fold diluted and centrifuged at 12,000 rpm for 10 min. The supernatant was filtered through a 0.45 µm nylon membrane and then injected into LC-ESI/MS for analysis. The concentration of IS was 1.00 µg/ml for all samples.

Three replicate injections were analysed to determine the extraction yields of astragalosides with the mean peak area.

2.4. Scanning electron microscopy (SEM)

A Hitachi S-520 field scanning electron microscopy (Hitachi, San Jose, CA, USA) was used to examine the morphological alteration of the dried sample with different extraction methods. After removing the solvent, the remaining Radix Astragali samples were plunged in liquid nitrogen and then cut with a cold knife. The sectioned particles were fixed on an adhesive tape and then sputtered with gold. All samples were examined with SEM under high vacuum condition at an accelerating voltage of 15.0 kV (20 μ m, 2000 \times magnification).

3. Results and discussion

3.1. Optimisation of MAE procedure

The factors concerning MAE include ethanol concentration, ratio of solid/liquid, irradiation time, extraction temperature, microwave irradiation power and extraction cycles. The influence of each

factor was studied by single-factor experiments. All assays were conducted in triplicate.

3.1.1. Effect of ethanol concentration

The selection of the most suitable solvent for extracting the analytes of interest from the sample matrix is a fundamental step in developing any extraction method. Methanol has a relatively higher dissipation factor, which means that it could absorb much of the microwave energy and transform it into heat better than other solvents. However, methanol was not tested in this study, because it is highly toxic and is not practical for use in food and pharmaceutical processing (Hemwimon et al., 2007). For this reason, mixtures of ethanol–water were tested. The rest of the variables employed were: solid/liquid ratio 1:20 (g/ml), three extraction cycles, each 10 min, extraction temperature 70 $^{\circ}$ C and microwave irradiation power 500 W.

From Fig. 3a, it can be observed that the yields of astragalosides were greatly influenced by the ethanol concentration. The yields of astragalosides increased sharply with the increase of ethanol concentration up to 80%. When extracted with 90% ethanol solution, the extraction yields decreased. From these results, it is clear that the addition of some amount of water improved the extraction efficiency. The possible reason for the increased efficiency might be

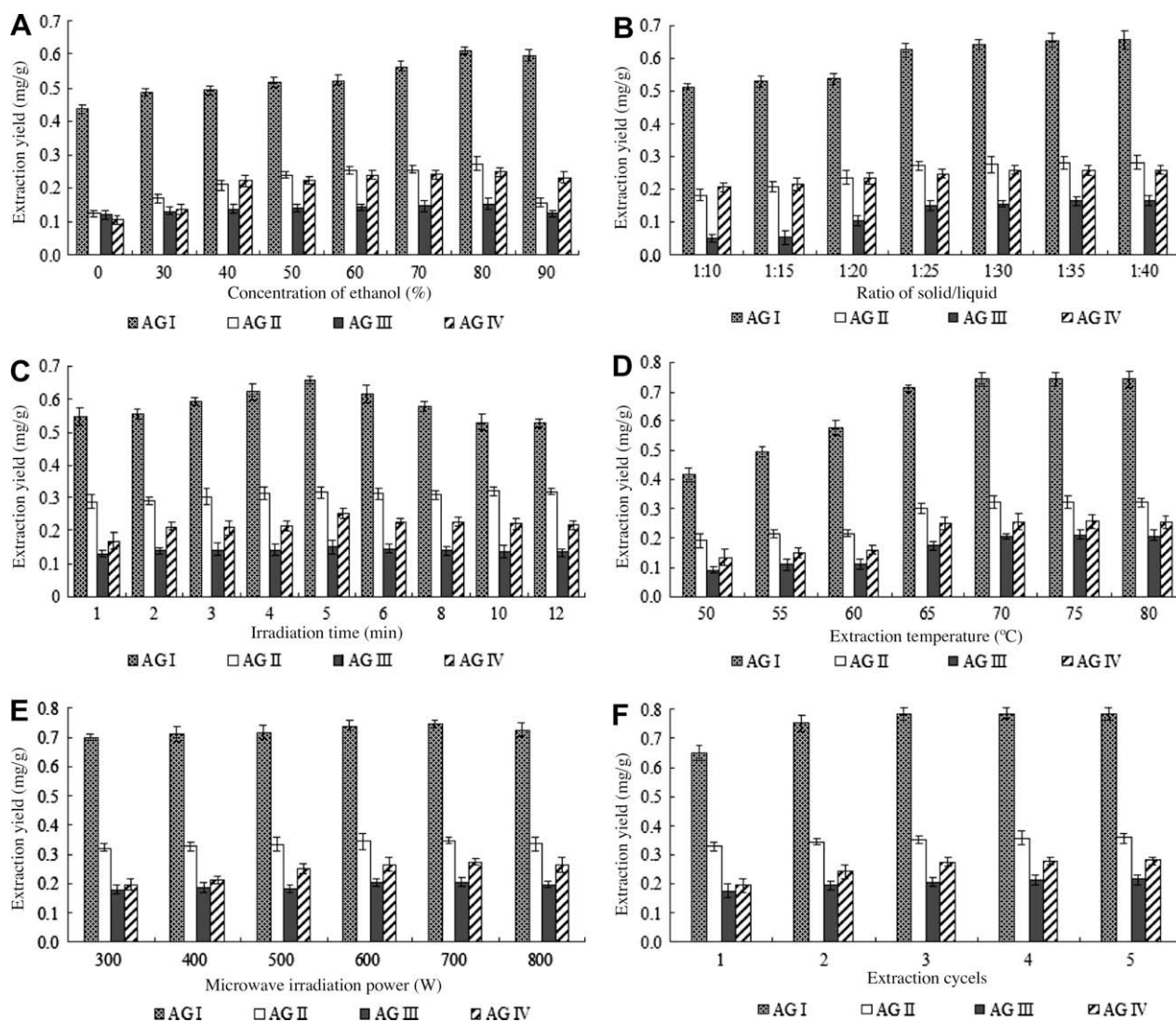


Fig. 3. Effect of different extraction parameters on the yields of four astragalosides in single-factor experiments.

due to the increase in swelling of plant materials by water, which increased the contact surface area between the plant matrix and the solvent (Li, Bo, Zhang, & Yao, 2004; Rostagno, Palma, & Barroso, 2003). Therefore, 80% ethanol solution was used in the following experiments.

3.1.2. Effect of ratio of solid/liquid

In investigating the influence of ratio of solid/liquid on yields of astragalosides, several tests were performed at different ratios of solid/liquid. The rest of the variables employed were: 80% ethanol as extraction solvent, three extraction cycles, each 10 min, extraction temperature 70 °C and microwave irradiation power 500 W. It can be seen in Fig. 3b that the yields of astragalosides increased with decreasing ratios of solid/liquid. In the tested range of 1:25–1:40 (g/ml), there was no significant difference, which was probably due to the larger volume of 80% ethanol solution causing excessive swelling of the materials by water and absorbing the effective constituent (Guo et al., 2001) or absorbing microwaves efficiently and very little microwave energy absorbed directly by the materials. Hence, a value of 1:25 (g/ml) was considered as the optimal ratio of solid/liquid for the MAE process, which can reduce processing costs.

3.1.3. Effect of irradiation time

It is necessary to select a proper irradiation time to guarantee completion of the extraction. Studies were carried out at different times, e.g., 1, 2, 3, 4, 5, 6, 8, 10 and 12 min. The rest of the extraction conditions were: 80% ethanol as extraction solvent, solid/liquid ratio 1:25 (g/ml), extraction temperature 70 °C, microwave irradiation power 500 W, and this process was repeated three cycles. As confirmed in Fig. 3c, with increasing irradiation times from 1 to 5 min, the extraction yields of astragalosides increased rapidly and reached its maximum at 5 min. Then, the extraction yields decreased with the extension of the irradiation time. This may be due to the decomposition of astragalosides at long irradiation time. Thus, 5 min was considered as the appropriate irradiation time.

3.1.4. Effect of extraction temperature

Extraction temperature is a factor that must be studied to increase the effectiveness of extraction of analytes employing microwave-assisted extraction. Generally, higher extraction temperature is profitable for the extraction and reduces the reaction time. To examine the performance of different extraction temperatures on the yields of astragalosides during MAE, an amount of 5.0 g materials were extracted with 80% ethanol solution for 5 min and a solid/liquid ratio of 1:25 (g/ml) under 500 W microwave irradiation power at different temperatures, repeated three cycles. Fig. 3d shows that the yields of astragalosides increased remarkably with increasing temperatures from 50 to 70 °C. Above 70 °C, the yields of astragalosides increased slowly and even decreased. Increasing temperatures may also cause an opening of the cell matrix, and as a result, improve extraction of astragalosides. Moreover, at high temperature, solvent viscosity decreased and the diffusivity increased, hence, the efficiency of extraction increased (Camel, 2000; Pan, Liu, Jia, & Shu, 2000). However, in order to avoid decomposition and scorching under high temperature, 70 °C was selected as the optimum temperature for extraction.

3.1.5. Effect of irradiation power

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions. Microwave irradiation energy can enhance the penetration of the solvent into the matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the sol-

vent and matrix, allowing the dissolution of components to be extracted.

In order to evaluate the effect of microwave irradiation power on MAE, the different microwave irradiation powers were controlled, e.g., 300, 400, 500, 600, 700, and 800 W. The rest of the extraction conditions were: 80% ethanol as extraction solvent, solid/liquid ratio 1:25 (g/ml), three extraction cycles and each 5 min under 70 °C extraction temperature. As shown in Fig. 3e, the high microwave irradiation power enhanced the yields of astragalosides when the power was lower than 700 W. However, when the power was higher than 700 W, the yields declined. This may be due to different matrixes having different appropriate microwave irradiation power. High irradiation power can offer superfluous energy to the solvent and matrix. It can drastically disturb molecular interactions. The unorderly molecular interaction structure can affect the extraction yields of astragalosides. Hence, 700 W was chosen as the appropriate microwave irradiation power.

3.1.6. Effect of extraction cycles

The effect of successive extractions of the residue, i.e., extraction cycles, was investigated in this experiment. The rest of the variables employed were: 80% ethanol as extraction solvent, solid/liquid ratio 1:25 (g/ml), extraction for 5 min, extraction temperature 70 °C and microwave irradiation power 700 W. The residue was taken back and re-extracted using fresh solvent each time. It can be observed from Fig. 3f, that the yields of astragalosides increased slowly without significant differences among the yields after three, four and five cycles. To save solvent, energy and time, three-cycle extraction is enough to release most of astragalosides into solvent. Three cycles were estimated as being sufficient to extract the four astragalosides present in *Radix Astragali*.

3.2. Extraction kinetics

In the extraction process, the extraction yield depends on both the extraction efficiency and chemical change of the target compound. The extraction efficiency is defined as the amount of the target compound transferred into the extraction solvent. The chemical structure of the target compound may change with some extraction conditions after the target compound being transferred into the extraction solvent, which can directly influence the extraction yield of the target compound (Chen et al., 2008). The extraction kinetics of four astragalosides under five extraction methods is shown in Fig. 4.

In MAE, with an increase of extraction time, the extraction yields of astragalosides I–IV increased in the initial 5 min, and then decreased from 5 to 20 min. Two possibilities may lead to these results. One is that the extraction of astragalosides I–IV was completed after 5 min. The other possibility is that the chemical structures of astragalosides may be changed by the loss of glucose or acetyl, which decreases the extraction yields. In SE, the yields of astragalosides I–IV increased with the extension of extraction time up to 240 min, and then showed no significant change. Using HRE, the yields of astragalosides reached its maximum in 60 min and did not significantly change from 60 to 120 min. In UE, the yields of four astragalosides increased with the increase of extraction time up to 40 min and remain constant, but the yields were lower than those obtained by MAE, SE and HRE. As for ME, it possessed a poor capability for the extraction of astragalosides, no clear increase of the yields of astragalosides was observed with increasing extraction times.

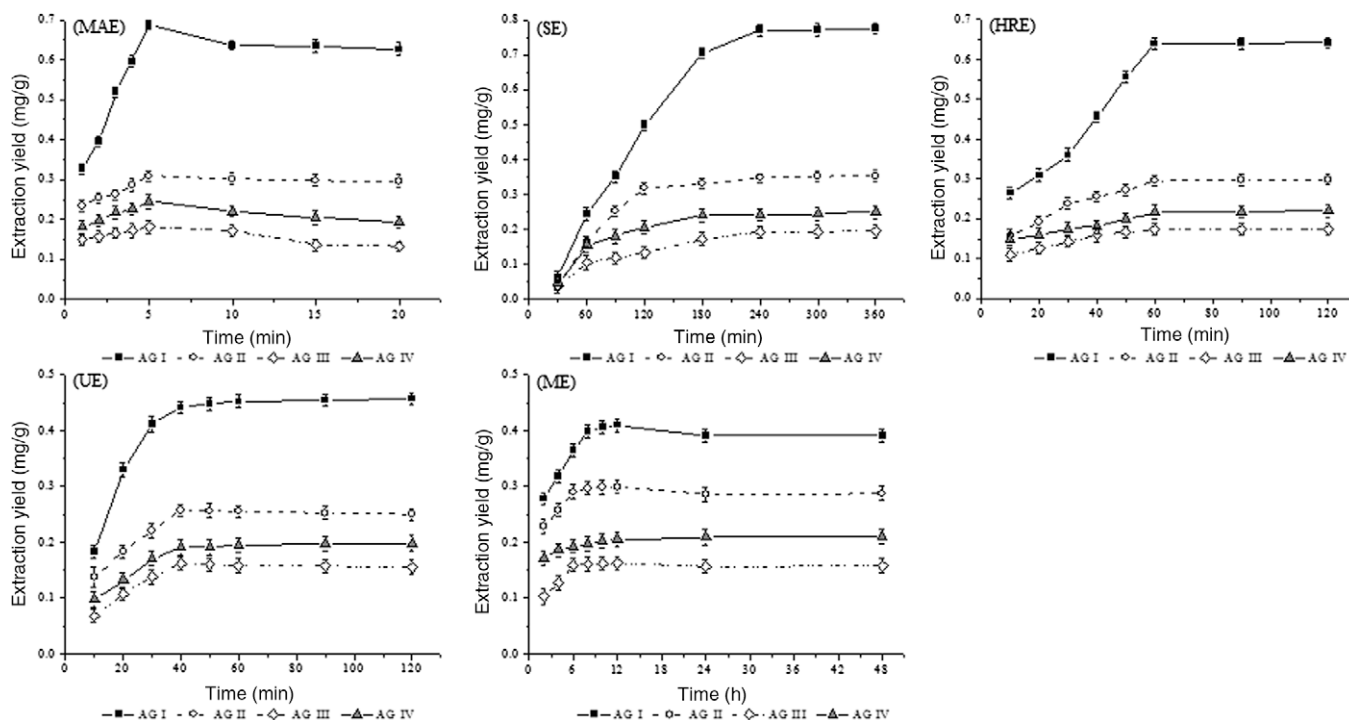


Fig. 4. Extraction kinetics of four astragalosides in Radix Astragali by different extraction techniques.

Table 1

Comparison of MAE and conventional extraction methods under the optimal conditions.

Method	AG I		AG II		AG III		AG IV	
	Yield (mg/g)	RSD (%)	Yield (mg/g)	RSD (%)	Yield (mg/g)	RSD (%)	Yield (mg/g)	RSD (%)
MAE	0.788	1.13	0.351	1.22	0.206	1.86	0.278	2.17
SE	0.770	1.68	0.347	2.27	0.193	1.73	0.242	1.95
HRE	0.761	2.19	0.352	1.94	0.203	2.01	0.257	2.32
UE	0.519	1.96	0.302	2.10	0.190	2.42	0.225	1.66
ME	0.411	1.77	0.299	1.46	0.166	1.82	0.206	2.11

3.3. Comparison of different extraction techniques

In the present study, MAE, SE, HRE, UE and ME techniques were compared for their extraction efficiency of four main astragalosides from Radix Astragali. The extraction yields of astragalosides obtained by five extraction methods under the optimal conditions are summarised in Table 1. The extraction time of MAE, SE, HRE, UE and ME were 15 min, 4, 3, 2 and 12 h, respectively. The extraction temperature of MAE, SE and HRE were 70, 90 and 90 °C, and the extraction temperature of UE and ME were room temperature.

The extraction yields of astragalosides obtained using MAE, HRE and SE methods were higher than those using UE and ME methods (Table 1). For AG I, AG II and AG III, similar yields were obtained using MAE, HRE and SE under different extraction conditions. The highest extraction yields using HRE and SE were obtained after 3 and 4 h extraction under 90 °C. While the extraction completeness by HRE and SE depended to a large extent on the extraction time and the extraction temperature, the completed extraction was achieved only in 15 min by MAE under 70 °C. In MAE, the target compounds can sufficiently absorb microwave energy and be quickly transferred into the extraction solvent, the extraction time was dramatically reduced and the extraction efficiency was considerably increased. Therefore, MAE represented a simple and efficient method for the extraction of astragalosides from Radix Astragali.

3.4. SEM observation

In order to study the structural alteration during the different extraction techniques and to understand the extraction mechanism, the plant samples were examined by scanning electron microscopy. Different extraction techniques produced distinguishable physical changes on Radix Astragali. Fig. 5 shows the micrographs of samples of raw materials (RM), MAE, SE, HRE, UE and ME, respectively. In MAE, the disruption on the surface of sample was obviously better than by other methods. The structure of cell was affected by microwave treatment due to the sudden temperature rise and the internal pressure increase. During this rupture process, the chemical substances within the cell are rapidly released into the surrounding extraction solvents. Moreover, the microwave energy penetrated the sample and solvent, and enhanced the extraction efficiency. In UE, the mechanical effect of ultrasound provided a greater penetration of solvent into cellular materials, via cavitation effects, and improved the release of chemical substances into the solvent. Hence, the surface of the sample after UE was obviously destroyed. In HRE and SE, the solvent transfers into the sample and extracts the compounds by permeation and solubilisation processed under higher temperatures. Hence, little destruction of the microstructure of sample occurs and a few of slight ruptures took place on the surface of the sample. In this process, more solvent, longer extraction time and higher

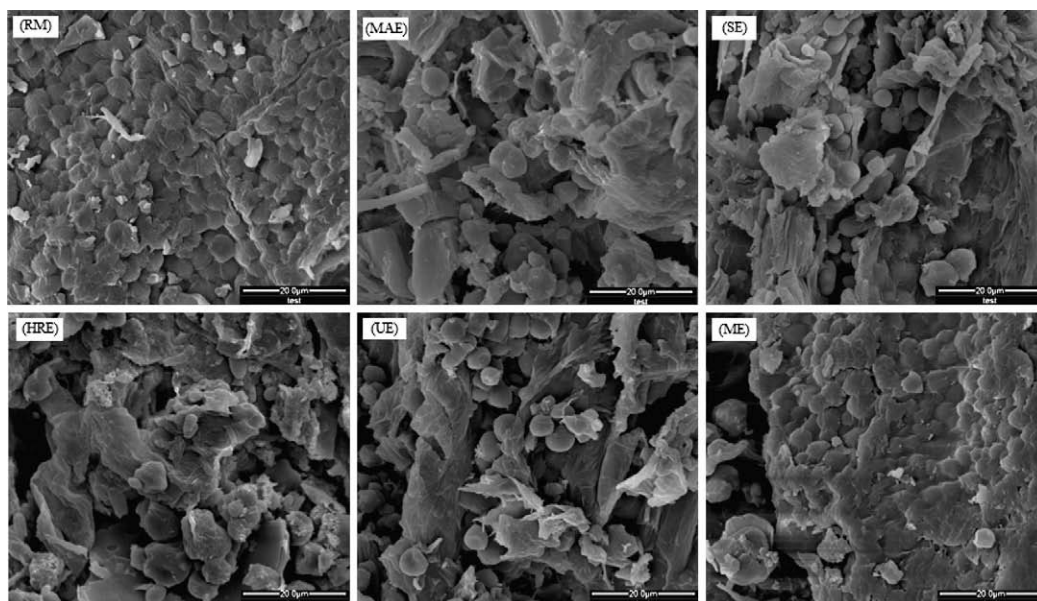


Fig. 5. SEM images (20.0 μm , 15.0 kV) of Radix Astragali after MAE, SE, HRE, UE and ME.

extraction temperature were needed. In ME, the surface of the sample was not considerably different from that of the raw materials, and only few slight creases were observed on the surface of the ME sample. Hence, the yields of astragalosides were the lowest among the different extraction techniques.

4. Conclusions

An efficient MAE method was developed for extraction of four astragalosides, i.e., AG I, AG II, AG III and AG IV from Radix Astragali. Astragalosides were directly quantified by LC-ESI/MS. To the best of our knowledge, this is the first report on combining MAE with LC-ESI/MS for extraction and quantification of astragalosides I–IV in Radix Astragali. Compared to conventional extraction techniques, MAE process required less extraction time and provided higher extraction efficiency. The main mechanism for the enhanced yields with MAE was the dipole rotation of the polar solvent in the microwave field, which was highly influenced by the solvent dielectric constant and dissipation factor. The optimum MAE conditions were extracted with 80% ethanol solution, ratio of solid/liquid of 1:25 (g/ml), at an extraction temperature of 70 °C, three extraction cycles, each 5 min under microwave irradiation power of 700 W. The results showed that MAE has an obvious predominance for the extraction of active compounds from Chinese herbs, especially triterpenoid saponins. Therefore, we conclude that MAE has a great potential for large-scale extraction of astragalosides from Radix Astragali. The results in this study can be referenced for the extraction of other compounds from herbal plants.

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