IF=1.545

Research article

Biomedical Chromatography

Received 2 October 2010, Revised 18 March 2011, Accepted 21 March 2011 Published online in Wiley Online Library: 28 April 2011

(wileyonlinelibrary.com) DOI 10.1002/bmc.1642

Evaluation of microwave-assisted extraction for aristolochic acid from Aristolochiae Fructus by chromatographic analysis coupled with nephrotoxicity studies

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ABSTRACT: In this paper, a microwave-assisted extraction (MAE) method was established for aristolochic acid-I from Aristolochiae Fructus, and the advantage of MAE was evaluated by chromatographic analysis coupled with nephrotoxicity studies. The experimental parameters of MAE for aristolochic acid-I in Aristolochiae Fructus were investigated and MAE was compared with Soxhlet extraction and ultrasound-assisted extraction in terms of extraction yields and extraction conditions. Under the optimum conditions, MAE could provide higher extraction yields of aristolochic acid-I (1.10 mg/g) than ultrasound-assisted extraction (0.82 mg/g) and Soxhlet extraction (0.95 mg/g), in addition to using less solvent and having a shorter extraction time. Furthermore, the nephrotoxicities of the extracts of Aristolochiae Fructus from different extraction procedures were investigated in Sprague–Dawley rats. The results of nephrotoxicity studies of, for example, general conditions, biochemistry parameters and histopathology examination showed no significantly differences in the nephrotoxicity levels of the extracts from MAE and that from Soxhlet extraction. These results indicated that MAE technique is a simple, rapid and effective extraction method, and the microwave irradiation during MAE procedure did not have any influence on the nephrotoxicity of Aristolochiae Fructus compared with Soxhlet extraction. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: microwave-assisted extraction; aristolochic acid; chromatographic analysis; nephrotoxicity; Aristolochiae Fructus

Introduction

Extraction is the first essential step for the isolation and analysis of target components from the natural products. There are many extraction methods, such as Soxhlet extraction (SE; de Castro and Priego-Capote, 2010), heating reflux extraction (HRE; Wu *et al.*, 2008), ultrasound-assisted extraction (UAE; Cao *et al.*, 2009), microwave-assisted extraction (MAE) (Tsubaki *et al.*, 2010) and supercritical fluid extraction (SFE; Macias-Sanchez *et al.*, 2009). MAE, a process utilizing the energy of microwaves to arouse molecular movement and rotation of liquids with a permanent dipole, leads to very fast heating of the solvent and sample. It provides considerable reductions in extraction time and solvent consumption with improved extraction rate and to some extent selective extraction. Therefore, MAE has been widely used in the extraction of biologically active compounds from natural products (Yan *et al.*, 2010; Xiao *et al.*, 2009a, b; Du *et al.*, 2009; Jiang *et al.*, 2009).

However, the traditional evaluation of MAE technique is always limited to chemical analysis for the determination of the target compounds. The indexes of different extraction techniques comparison are also limited to extraction conditions and extraction yields. It is not clear whether microwave irradiation influences the integrated activities of the natural products during MAE or not. Some researchers have implied that different extraction procedures might have different influences on the activities of the natural products, such as antioxidant activity (Zigoneanu *et al.*, 2008; Kalia *et al.*, 2008; Hayat *et al.*, 2009; Sharma *et al.*, 2008) and antimicrobial activity (Bendahou *et al.*, 2008; Okoh *et al.*, 2010). Zigoneanu *et al.* studied the extraction and the antioxidant activity of rice bran oil and found that there was no difference in the antioxidant activity of oil between MAE and solvent extraction at 40°C (Zigoneanu *et al.*, 2008). Kalia *et al.* discovered that the Trolox equivalent antioxidant capacity of Soxhlet extracts from *Potentilla atrosanguinea* was 1.8 and 3 times higher than those obtained by UAE and maceration, but only slightly (1.2 times) higher than that obtained by MAE (Kalia *et al.*, 2008). Bendahou *et al.* studied the antimicrobial activity and chemical composition of essential oil obtained from *Origanum glandulosum* Desf. and found that *Origanum glandulosum* oil obtained by solvent-free MAE has both antimicrobial and antifungal activities, while the *Origanum glandulosum* oil obtained

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Abbreviations used: AA-I, Aristolochic acid-I; BUN, blood urea nitrogen; HRE, heating reflux extraction; MAE, microwave-assisted extraction; Scr, serum creatinine; SE, Soxhlet extraction; SFE, supercritical fluid extraction; UA, uric acid; UAE, ultrasound-assisted extraction; UP, urine protein.

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by hydrodistillation only has antifungal activity (Bendahou *et al.*, 2008). To our knowledge, there is no report about the influence of the extraction procedure on the pharmacological actions of natural products. Therefore, studies on the influence of microwave irradiation during MAE on the pharmacological actions of the natural products are very important.

Aristolochiae Fructus (Madouling in Chinese), the dried fruit of *Aristolochiae contorta* or *Aristolochiae debilis*, is a traditional Chinese medicine and has been extensively used in China and Korea as a remedy for hemorrhoids, coughs and asthma (Hwang *et al.*, 2006). Aristolochic acid-I [AA-I; 8-methoxy-6-nitro-phenanthro (3, 4-*d*)-1, 3-dioxolo-5-carboxylic acid; Fig. 1] is the major component in Aristolochiae Fructus and has been discovered to have severe nephrotoxic effects (Debelle *et al.*, 2008; Lord *et al.*, 1999; Levi *et al.*, 1998). All of the extraction methods reported for the analysis of AA-I in Aristolochiae Fructus, including SFE (Liang *et al.*, 2010), UAE (Yuan *et al.*, 2008; Chan *et al.*, 2007; Wei and Feng, 2008), SE (Liu *et al.*, 2005) and HRE (Zhai *et al.*, 2006), require long extraction times and large amounts of organic solvents, and are labor-intensive. The aim of this study was to establish an efficient MAE method of AA-I extraction from Aristolochiae Fructus and to study the influence of microwave irradiation during MAE on the nephrotoxicity of Aristolochiae Fructus.

In this work, the predominance of MAE for AA-I from Aristolochiae Fructus was evaluated by chromatographic analysis coupled with nephrotoxicity studies. The experimental parameters of MAE such as extraction solvent, extraction temperature, extraction time and ratio of liquid to solid were investigated. MAE for AA-I was also compared with other methods in terms of extraction yield, extraction time and solvent consumption. Furthermore, the nephrotoxicity of the extracts of Aristolochiae Fructus from different extraction procedures were investigated in Sprague–Dawley rats. The results might provide useful information on the influence of microwave irradiation on the integrated activities of the natural products during MAE procedure.

Experimental

Materials and reagents

Dried Aristolochiae Fructus was purchased from the Caizhiling Medicinal Material Emporium in Guangzhou. The materials were triturated and passed through a 20 mesh stainless steel sieve, then stored in a closed desiccator. The same batch of sample was used for the experiments.

Acetonitrile of HPLC grade, used for the mobile phase, was purchased from Merck (Darmstadt, Germany). Standard of AA-I was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Other reagents were of analytical grade and were purchased from Guangzhou Chemical Reagents Factory (Guangzhou, China). Distilled water was used throughout the study.

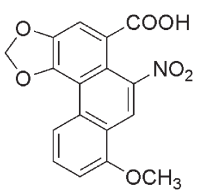

Figure 1. The chemical structure of AA-I.

Apparatus

MAE experiments were performed with an MAS-II microwave oven (Sineo Microwave Chemistry Technology Company, Shanghai, China) with a frequency of 2450 MHz and a maximum delivered power of 1000 W. UAE was performed with an AS3120 Ultrasonic Cleaner (Tianjin Automatic Science Instrument Co. Ltd., Tianjin, China) with an output power of 120 W and a frequency of 40 kHz. The Soxhlet extractor comprised a 100 mL Soxhlet thimble, a 500 mL round-bottom flask containing extraction solvent and a boiling regulator. The concentration of the sample was determined with an RE52CS-1 rotary evaporator (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) coupled with a B-260 thermostatic water bath (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) for heating.

HPLC analysis was carried out on an HPLC integrated system LC-2010C_{HT} (Shimadzu, Japan) which consists of an SCL-10Avp system controller, two LC-10ATvp pumps, an SPD-10Avp UV–vis detector and a model 7725 injection valve furnished with a 20 μL loop. The chromatographic data were recorded and processed with the Class-VP Workstation software (Shimadzu, Japan).

Preparation of standard solutions

The stock solution of AA-I (100 mg/L) was prepared in acetonitrile. Standard solutions of AA-I with concentrations of 50, 40, 30, 20, 10, 5.0, 2.0, 1.0, 0.50 and 0.25 mg/L were made by serial dilutions of AA-I stock solution with acetonitrile. All the solutions were stored at 1–4°C in darkness.

Extraction of AA-I from Aristolochiae Fructus

The operational conditions of each method described below were optimum as assessed by the highest yield determined through experimental design. The extraction yield of AA-I (mg/g) was defined as follows:

$$\text{Yield}(\text{mg/g}) = \frac{\text{mass of AA-I in the extraction solution}}{\text{mass of Aristolochiae Fructus powder taken}} \times 1000$$

Microwave-assisted extraction. Sample of 3.0 g was extracted by MAE using 45 mL of 50% (v/v) methanol aqueous solution. The extraction time was 5 min and the extraction temperature was 70°C for MAE. The MAE conditions were optimized according to an orthogonal design $L_9(3^4)$ combined with a mono-factor test. The orthogonal design extraction experiment was carried out with three factors and three levels, namely extraction temperature (50, 60 and 70°C), extraction time (5, 10 and 15 min) and ratio of liquid to solid (10:1, 15:1 and 20:1). The range of each factor level was based on the results of preliminary experiments. All extraction experiments were repeated three times. The extracts obtained were filtered and then were diluted to 100 mL with the extraction solvent. The mass of AA-I in the extraction solution (one-step extraction) was analyzed by RP-HPLC.

Under the optimized extraction conditions, the extraction of MAE was scaled up 10-fold and the sample (30.0 g) was extracted. Then the extracts were filtered and concentrated to dryness by a rotary evaporator under reduced pressure. The crude extracts were stored at −20°C until further use in the nephrotoxicity study.

Soxhlet extraction. A 3.0 g sample of Aristolochiae Fructus was put into a Soxhlet thimble. The flask was filled with 225 mL methanol and heated at 75°C in a water bath, then the solvent was refluxed for 8 h. The extracts obtained were filtered and then were diluted to 250 mL with the extraction solvent. The mass of AA-I was analyzed by HPLC.

In the nephrotoxicity study, the extraction of SE was scaled up by 10-fold and the sample (30.0 g) was extracted. Then the extracts were filtered and were concentrated to dryness by a rotary evaporator under reduced pressure. The crude extracts were stored at −20°C until further use.

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Ultrasound-assisted extraction. Sample powder 3.0 g was placed into a conical flask. After adding 120 mL of methanol, the flask was sonicated for 35 min in an ultrasonic bath. The extracts obtained were filtered and then were diluted to 200 mL with the extraction solvent. The mass of AA-I was analyzed by HPLC.

Chromatographic conditions

Chromatographic separations were performed on a Diamonsil C_{18} column (250 × 4.6 mm i.d., 5 µm, Dikma, China), equipped with an EasyGuard C_{18} guard column (10 × 4.6 mm i.d., Dikma, China) at 26°C. The mobile phase was composed of acetonitrile–0.5% acetic acid aqueous solution (58:42, v/v). The flow rate was 1.0 mL/min, the injection volume was 10 µL and the detection wavelength was set at 250 nm.

The Nephrotoxicity study of Aristolochiae Fructus extracts from MAE and SE

Animals and environment. The specific pathogen-free Sprague–Dawley rats used in this study were obtained from the Experimental Animal Center of Guangzhou University of Chinese Medicine (permission no. 0040812). Six-week-old female Sprague–Dawley rats were used after a 7 day acclimatization period. Rats were kept in a fully air-conditioned animal room with a natural light/dark cycle. The temperature was maintained at 20 ± 2°C with a relative humidity of 50 ± 20%. The rats were given *ad libitum* access to food and water. The study in animals was in accordance with ethical guidelines stated in the Guide for the Care and Use of Laboratory Animals, approved by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, China.

Fourteen-day nephrotoxicity study in Sprague–Dawley rats. Three groups of rats were used, each consisting of 10 SD rats. Two of them were given the extracts of Aristolochiae Fructus obtained from MAE and SE, respectively, for 14 days. The control group was given distilled water 10 mL/kg for 14 days. In this study, a single 15 g/kg (crude drug/body weight) daily dose of the extracts were given to the rats by intragastric administration, which was just below the dose (18 g/kg) used in the literature (Hwang *et al.*, 2006). The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured daily. At the end of 14 days, the blood samples from the oculi chorioidea vein of the rats and the urine samples were collected for biochemical determinations. Biochemical determinations were performed with a Hitachi 7060 automatic biochemical analyzer (Hitachi, Japan).

Histopathological examination. Tissue samples from the kidneys were excised, fixed in formalin and prepared for histological examination. Paraffin sections were stained with hematoxylin and eosin (HE) and were examined microscopically.

Statistical analysis

Statistical analysis of biochemistry parameters such as blood urea nitrogen (BUN), serum creatinine (Scr), urine protein (UP) and uric acid (UA) was carried out by one-way analysis of variance test. The results are expressed as mean values ± standard deviation, and the means were compared using Student's *t*-test. *p*-Values of <0.05 were considered significant.

Results and discussion

Investigation of the extraction method

Investigation of MAE procedure. In this study, several influential extraction parameters of MAE such as solvent type, solvent composition, preleaching time, extraction temperature, extraction time and liquid–solid ratio were systematically studied to obtain the maximum yield of AA-I.

Ethyl acetate, ethanol, water, methanol, 30% (v/v) methanol aqueous solution, 50% (v/v) methanol aqueous solution and 80% (v/v) methanol aqueous solution were investigated as extraction solvents. A 50% (v/v) methanol aqueous solution showed the best extraction efficiency because of its good microwave-absorptive property and the high solubility of AA-I. Therefore, 50% (v/v) methanol aqueous solution was chosen for further application.

Sufficient soakage of the herb, which helps the bursting of the cell wall, could enhance the extraction yield for the herb matrix when it is free of water. However, there was little increase in the extraction yield after pre-leaching of 10 h. This was probably because the high polarity extraction solution composed of 50% (v/v) methanol aqueous solution could swell the herb material rapidly under microwave radiation.

The optimization of extraction parameters such as extraction temperature, extraction time and liquid–solid ratio was investigated with an orthogonal design L_9 (3^4). The results of the experiment, shown in Table 1, indicated that the maximum extraction yield of AA-I was 1.10 mg/g. Comparing the *R*-values, the influence of factors on the mean extraction yield of AA-I decreased in the order extraction temperature, extraction time and liquid–solid ratio. Thus, the optimum experimental conditions were obtained: extraction temperature, 70°C; extraction time, 5 min; and ratio of liquid–solid, 15:1 (the seventh set of experimental extraction conditions; see Table 1).

Investigation of MAE-HPLC method. The AA-I in the sample was identified by its chromatogram and by its retention time in comparison with that of the authentic standard compound. Representative chromatograms of standard solution of AA-I and extraction solution obtained from Aristolochiae Fructus by MAE are shown in Fig. 2. The retention time for AA-I was 10.6 min and there was no significant interference observed at the retention time of the analyte.

The linearity, limit of detection, reproducibility and recovery of the MAE-HPLC method were investigated. The calibration curves, which related the concentrations of AA-I to the peak areas of AA-I, showed good linearity over the range 0.25–50 mg/L ($R = 0.9998$). The limit of detection was 0.075 mg/L, which was evaluated on the basis of a signal-to-noise ratio of 3.0. The reproducibility was estimated by five repetitive samples extracted by MAE at the optimum conditions, and the relative standard derivation (RSD) was lower than 1.9%. The recovery of AA-I under the optimum conditions was evaluated by the standard addition method at 1.0 mg/g. The recovery for AA-I was 89.0%, with an RSD lower than 2.0%. The results proved that the method had good precision and accuracy.

The present method was applied for the determination of AA-I in Aristolochiae Fructus from different provinces, including Liaoning, Hebei, Neimenggu, Anhui, Henan, Zhejiang and Heilongjiang. The concentrations of AA-I were in the range 0.61-1.04 mg/g and the corresponding RSDs were less than 4.1%. The results indicated that the concentrations of AA-I in Aristolochiae Fructus from different regions varied greatly, and the MAE-HPLC method was feasible for extracting and analyzing AA-I from Aristolochiae Fructus.

The advantage of MAE evaluated by chromatographic analysis

The advantage of an extraction method could be evaluated by the advantages and disadvantages of the process, such as

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Table 1. Experimental conditions and different extraction yields[a] extracted with the orthogonal design L_9 (3^4) ($n = 3$)

	Factors				
No.	Extraction temperature (°C)	Extraction time (min)	Liquid–solid ratio (mL/g)	Yield (mg/g)	RSD (%)
1	50	5	10:1	0.92	1.0
2	50	10	15:1	0.90	2.0
3	50	15	20:1	0.95	0.4
4	60	5	20:1	0.95	2.1
5	60	10	10:1	1.00	0.4
6	60	15	15:1	1.04	1.1
7	70	5	15:1	1.10	0.7
8	70	10	20:1	1.08	0.6
9	70	15	10:1	1.08	0.6
K_1	0.923	0.990	1.000		
K_2	0.997	0.993	1.013		
K_3	1.087	1.023	0.993		
R	0.164	0.033	0.020		

[a]Each extraction yield is the mean of three independent experiments.
$K_i^A = \Sigma$(yield of AA-I at A_i)/3; $R_i^A = \max\{K_i^A\} - \min\{K_i^A\}$.

extraction yield, complexity, production cost, environmental friendliness and safety. To evaluate the predominance of MAE, UAE and SE were also applied for the extraction of AA-I from Aristolochiae Fructus in this study. All of the extraction techniques were operated at their optimum conditions. Table 2 shows the comparison of extraction yields and extraction conditions of the three extraction methods for AA-I from Aristolochiae Fructus.

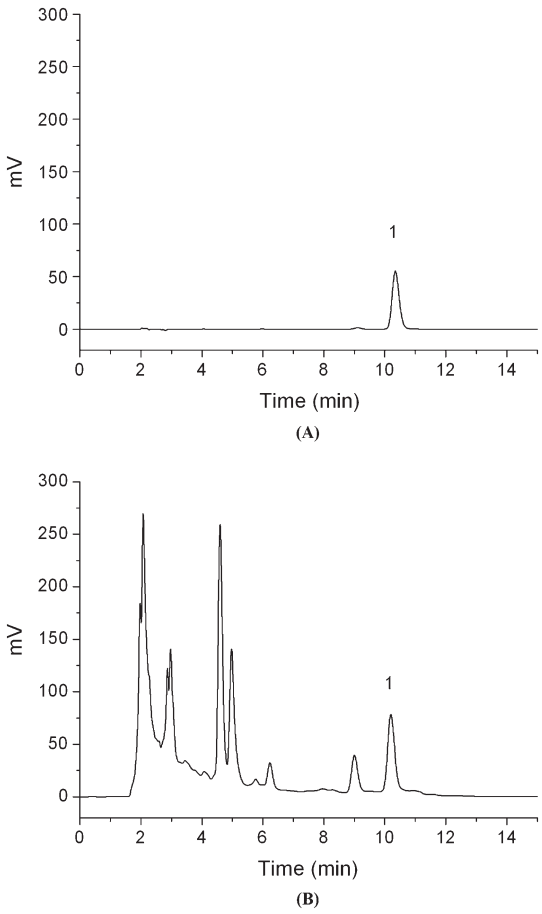

Figure 2. HPLC chromatograms: (A) standard solution of 20 mg/L AA-I; (B) extraction solution obtained from Aristolochiae Fructus by MAE. Peak **1** is AA-I.

The extraction yield of MAE for AA-I (1.10 mg/g) was much higher than that of UAE (0.82 mg/g) and was slightly higher than that of SE (0.95 mg/g). The extraction times of UAE and that of SE were 35 and 480 min, respectively, while that of MAE was only 5 min. The liquid–solid ratios of UAE and SE were 40:1 and 75:1, respectively, while that of MAE was only 15:1. Compared with UAE and SE, MAE could provide higher extraction yields by using less solvent at shorter extraction times, indicating that MAE is a rapid, effective and environmentally friendly sample preparation technique. The advantage of MAE was due to its unique extraction mechanisms. During MAE procedure, microwaves directly heat solvents and samples; therefore, the direct interaction of microwaves with the extraction solutions present in the cells results in the subsequent rupture of the cells and release of intracellular products into the solvent.

Although the predominance of MAE was demonstrated by chromatographic analysis, the nephrotoxicity of the extract might be influenced by microwave irradiation during MAE. Further experiments investigating the influence of extraction method on the nephrotoxicity of Aristolochiae Fructus should based on different extraction procedures and the same content of the nephrotoxic component. Since the extraction yield of AA-I in SE was close to that in MAE, SE was chosen to compare with MAE in the nephrotoxicity studies.

Table 2. Comparison of three extraction methods for the extraction of AA-I from Aristolochiae Fructus ($n = 3$)

Extraction method	Liquid–solid ratio (mL/g)	Extraction time (min)	Yield of AA-I (mg/g)	RSD (%)
MAE	15:1	5	1.10	0.7
UAE	40:1	35	0.82	1.9
SE	75:1	480	0.95	3.3

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Table 3. The results of biochemistry parameters and terminal body weight ($n = 10$)

Group	BUN (mmol/L)	Scr (μmol/L)	UP for 24 h (mg)	UA for 24 h (μmol)	Body weight (g)
MAE	6.36 ± 2.09	61.6 ± 4.3	55.9 ± 18.0*	8.35 ± 2.10	194 ± 7
SE	5.14 ± 0.51	61.8 ± 2.7	62.1 ± 17.3*	6.60 ± 1.97	169 ± 8*
Control	5.65 ± 0.87	58.2 ± 4.1	26.6 ± 10.6	7.82 ± 1.75	196 ± 11

*$p < 0.05$, compared with the control group.

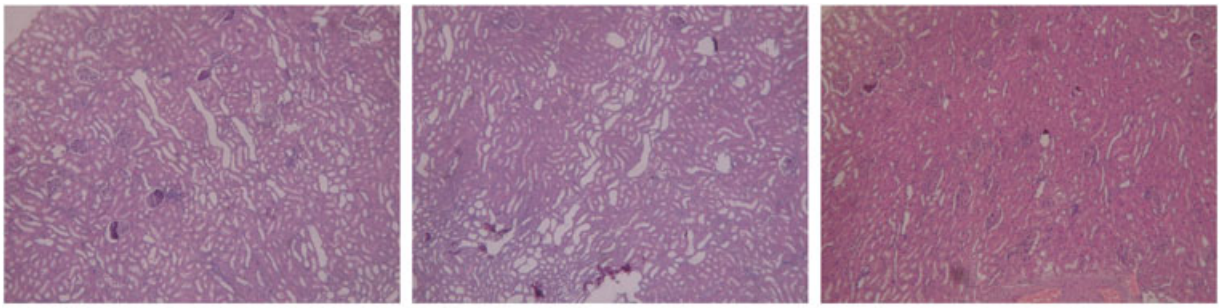

Figure 3. The histopathological examination of the kidney: (A) the group of MAE; (B) the group of SE; (C) the control group (hematoxylin and eosin, ×100).

The influences of different extraction procedures on the nephrotoxicity of Aristolochiae Fructus

AA-I is the major component in Aristolochiae Fructus, and has been discovered to have significantly nephrotoxicity (Hwang *et al.*, 2006; Debelle *et al.*, 2008; de Jonge and Vanrenterghem, 2008). It has been reported that the kidney is the toxic target and the mechanism of nephrotoxicity of AA-I is renal tubular epithelium necrosis, apoptosis and renal interstitial fibrosis (Lord *et al.*, 1999; Stiborova *et al.*, 2008). In this study, the nephrotoxicity of the extracts of Aristolochiae Fructus obtained from MAE and SE was compared to evaluate the influence of different extraction procedures on the nephrotoxicity of Aristolochiae Fructus. The extractions of MAE and SE were scaled up 10-fold. The extraction yield of AA-I obtained from scaled up MAE (0.75 mg/g) was close to that of scaled up SE (0.73 mg/g), while the operation conditions of the two methods, such as the extraction solvent, extraction time and the ratio of liquid–solid, were different. The nephrotoxicities of the extracts of Aristolochiae Fructus obtained from MAE and SE were compared as described below.

Cage side observation and body weight measurements. At an early stage, autopsy showed some casualties caused by technical errors in tube feeding instead of drug toxicity. At a late stage of dosing, the animals in the MAE and SE groups showed depression, reduced movement, erect hair and sitting in a head-up position. Reduced body weight gain was observed in the group of SE, but there were no significant differences in the body weight gain of the MAE group compared with the control group (see Table 3). Survival was not affected by the treatment with the extracts of Aristolochiae Fructus. These phenomena and clues are in accordance with the literatures (Hwang *et al.*, 2006; Hu *et al.*, 2004) and suggested that the toxicity of MAE and SE groups was stronger than that of the control group.

Biochemistry parameters. Several main biochemistry parameters such as BUN, Scr, UP and UA were analyzed (see Table 3). Generally, the increases in BUN and Scr suggest glomerular damage, and the increases in UP and UA indicate nephritis. However, the results in Table 3 show that there were no significant changes in BUN, Scr and UA levels in the testing groups in comparison with the control group. This meant that the results of BUN, Scr and UA did not reveal any toxicologically significant changes, which is in accordance with the literature (Hwang *et al.*, 2006). The UP result showed that, after taking the herbal extracts for 2 weeks, there was a significant increase in UP level in the testing groups in comparison with the control group ($p < 0.05$), but there was no significant difference between the MAE and SE groups.

Histopathological examination. HE staining (Fig. 3) showed significant renal tubular dilation in both MAE and SE groups after 14 days of treatment. However, renal tubular dilation was not shown in the control group. Compared with the control group, renal injury was observed in both MAE and SE groups, but there was no significant difference between the two groups.

According to the results shown above, the extracts of MAE and SE from Aristolochiae Fructus indicated significant nephrotoxicity, which was not shown in the control group. There were also a few differences in the nephrotoxicity levels between the extracts obtained from MAE and SE techniques. Therefore, the same nephrotoxicity level of the extracts obtained from MAE and SE, which had almost equal contents of AA-I, demonstrated that the microwave irradiation during MAE procedure did not have any influence on the nephrotoxicity of Aristolochiae Fructus compared with the traditional extraction method.

Conclusions

In this study, a simple and rapid MAE method was developed and validated for the extraction of AA-I from Aristolochiae Fructus, and the advantage of MAE was evaluated by chromatographic analysis combined with nephrotoxicity studies.

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Compared with UAE and SE, MAE could provide higher extraction yields in addition to using less solvent and having a shorter extraction time. Furthermore, the nephrotoxicity studies showed that there was no significant difference between the nephrotoxicity levels of extracts from MAE and those from SE. The results indicated that MAE technique is a simple, rapid and effective extraction method with no influence the integrated activity such as the nephrotoxicity of Aristolochiae Fructus compared with the traditional extraction technique.

Acknowledgments

This work was supported by the National Key Project of Scientific and Technical Supporting Programs Funded by Ministry of Science and Technology of China (grant no. 2006BAK03A08), the Natural Science Foundation of China (grant no. 20905080) and the Guangdong Provincial Department of Science and Technology of China (no. 2009B010900021).

References

Bendahou M, Muselli A, Grignon-Dubois M, Benyoucef M, Desjobert JM, Bernardini AF and Costa J. Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. essential oil and extract obtained by microwave extraction: comparison with hydro distillation. *Food Chemistry* 2008; **106**: 132–139.

Cao X, Ye X, Lu Y, Yu Y and Mo W. Ionic liquid-based ultrasonic-assisted extraction of piperine from white pepper. *Analytica Chimica Acta* 2009; **640**: 47–51.

Chan W, Lee KC, Liu N and Cai Z. A sensitivity enhanced high-performance liquid chromatography fluorescence method for the detection of nephrotoxic and carcinogenic aristolochic acid in herbal medicines. *Journal of Chromatography A* 2007; **1164**: 113–119.

de Castro MDL and Priego-Capote F. Soxhlet extraction: past and present panacea. *Journal of Chromatography A* 2010; **1217**: 2383–2389.

de Jonge H and Vanrenterghem Y. Aristolochic acid: the common culprit of Chinese herbs nephropathy and Balkan endemic nephropathy. *Nephrology, Dialysis, Transplantation* 2008; **23**: 39–41.

Debelle FD, Vanherweghem J and Nortier JL. Aristolochic acid nephropathy: a worldwide problem. *Kidney International* 2008; **74**: 158–169.

Du FY, Xiao XH, Luo XJ and Li GK. Application of ionic liquids in the microwave-assisted extraction of polyphenolic compounds from medicinal plants. *Talanta* 2009; **78**: 1177–1184.

Hayat K, Hussain S, Abbas S, Farooq U, Ding B, Xia S, Jia C, Zhang X and Xia W. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity *in vitro*. *Separation and Purification Technology* 2009; **70**: 63–70.

Hu SL, Zhang HQ, Chan K and Mei QX. Studies on the toxicity of *Aristolochia manshuriensis* (Guanmuton). *Toxicology* 2004; **198**: 195–201.

Hwang MS, Park MS, Moon JY, Lee JS, Yum YN, Yoon E, Lee H, Nam KT, Lee BM, Kim SH and Yang KH. Subchronic toxicity studies of the aqueous extract of *Aristolochiae fructus* in Sprague–Dawley rats. *Journal of Toxicology and Environmental Health – Part A* 2006; **69**: 2157–2165.

Jiang Z, Liu F, Goh JJL, Yu L, Li SFY, Ong ES and Ong CN. Determination of senkirkine and senecionine in *Tussilago farfara* using microwave-assisted extraction and pressurized hot water extraction with liquid chromatography tandem mass spectrometry. *Talanta* 2009; **79**: 539–546.

Kalia K, Sharma K, Singh HP and Singh B. Effects of extraction methods on phenolic contents and antioxidant activity in aerial parts of *Potentilla atrosanguinea* Lodd. and quantification of its phenolic constituents by RP-HPLC. *Journal of Agricultural and Food Chemistry* 2008; **56**: 10129–10134.

Levi M, Guchelaar HJ, Woerdenbag HJ and Zhu YP. Acute hepatitis in a patient using a Chinese herbal tea a case report. *Pharmacy World & Science* 1998; **20**: 43–44.

Liang Q, Chow A, Wang Y, Tong H and Zheng Y. Removal of toxic aristolochic acid components from Aristolochia plants by supercritical fluid extraction. *Separation and Purification Technology* 2010; **72**: 269–274.

Liu LF, Cao SP, Ji Y, Zhang XJ and Huang T. Qualitative and quantitative analysis of aristolochic acids in Chinese materia medica and traditional Chinese patent medicines. *Chinese Traditional Patent Medicine* 2005; **27**: 938–941.

Lord GM, Tagore R, Cook T, Gower P and Pusey CD. Nephropathy caused by Chinese herbs in the UK. *Lancet* 1999; **354**: 481–482.

Macias-Sanchez MD, Mantell C, Rodriguez M, de la Ossa EM, Lubian LM and Montero O. Comparison of supercritical fluid and ultrasound-assisted extraction of carotenoids and chlorophyll a from *Dunaliella salina*. *Talanta* 2009; **77**: 948–952.

Okoh OO, Sadimenko AP and Afolayan AJ. Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis* L. obtained by hydrodistillation and solvent free microwave extraction methods. *Food Chemistry* 2010; **120**: 308–312.

Sharma UK, Sharma K, Sharma N, Sharma A, Singh HP and Sinha AK. Microwave-assisted efficient extraction of different parts of *Hippophae rhamnoides* for the comparative evaluation of antioxidant activity and quantification of its phenolic constituents by reverse-phase high-performance liquid chromatography (RP-HPLC). *Journal of Agricultural and Food Chemistry* 2008; **56**: 374–379.

Stiborova M, Frei E and Schmeiser HH. Biotransformation enzymes in development of renal injury and urothelial cancer caused by aristolochic acid. *Kidney International* 2008; **73**: 1209–1211.

Tsubaki S, Sakamoto M and Azuma J. Microwave-assisted extraction of phenolic compounds from tea residues under autohydrolytic conditions. *Food Chemistry* 2010; **123**: 1255–1258.

Wei F and Feng YQ. Rapid determination of aristolochic acid I and II in medicinal plants with high sensitivity by cucurbit[7]uril-modifier capillary zone electrophoresis. *Talanta* 2008; **74**: 619–624.

Wu CH, Murthy HN, Hahn EJ, Lee HL and Paek KY. Efficient extraction of caffeic acid derivatives from adventitious roots of *Echinacea purpurea*. *Journal of Food Sciences* 2008; **26**: 254–258.

Xiao X, Guo Z, Deng J and Li G. Separation and purification of isofraxidin from *Sarcandra glabra* by microwave-assisted extraction coupled with high-speed counter-current chromatography. *Separation and Purification Technology* 2009a; **68**: 250–254.

Xiao XH, Wang JX, Wang G, Wang JY and Li GK. Evaluation of vacuum microwave-assisted extraction technique for the extraction of antioxidants from plant samples. *Journal of Chromatography A* 2009b; **1216**: 8867–8873.

Yan MM, Liu W, Fu YJ, Zu YG, Chen CY and Luo M. Optimisation of the microwave-assisted extraction process for four main astragalosides in Radix Astragali. *Food Chemistry* 2010; **119**: 1663–1670.

Yuan J, Liu Q, Zhu W, Ding L, Tang F and Yao S. Simultaneous analysis of six aristolochic acids and five aristolactams in herbal plants and their preparations by high-performance liquid chromatography–diode array detection–fluorescence detection. *Journal of Chromatography A* 2008; **1182**: 85–92.

Zhai ZD, Luo XP and Shi YP. Separation and determination of aristolochic acids in herbal medicines by microemulsion electrokinetic chromatography. *Analytica Chimica Acta* 2006; **561**: 119–125.

Zigoneanu IG, Wilhams L, Xu Z and Sabliov CM. Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. *Bioresource Technology* 2008; **99**: 4910–4918.

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