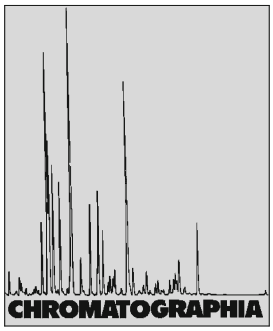
Dynamic Microwave-Assisted Extraction of Arctigenin from *Saussurea medusa* Maxim.



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Abstract

Dynamic microwave-assisted extraction (DMAE) technique was used for the fast extraction from *Saussurea medusa* Maxim. In order to achieve the optimal extraction conditions, variables involved in the extraction procedure such as extraction methods, extraction solvents, methanol concentration, ratio of solvent to raw material, extraction time, microwave power and extraction cycles were investigated. Orthogonal L_9 $(3)^4$ test design in the extraction mode was used for optimization extraction conditions and the maximum content of arctignenin was 10.891 ± 0.003 mg g^{-1} obtained by once DMAE at 390 W with 50 mL methanol for 20 min. The extraction efficiency of arctigenin with DMAE was higher than other traditional extraction methods.

Keywords

Column liquid chromatography
Microwave-assisted extraction
Arctigenin
Saussurea medusa Maxim.

Introduction

Saussurea medusa Maxim. is a rare materia medica grown in Gansu, Qinghai, Sichuan, Yunnan, Tibet province of China. The whole plant of *S. medusa* is widely used for the treatment of rheumatic arthritis, stroke, anthrax, febricity, detumescence and gynopathy [1, 2]. Some studies have been reported on the composition of *S. medusa*. The main constituents are flavonoids, flavonoid glycosides, lignans, coumarins, essential oil, polysaccharides and other substances [3–10]. Arctigenin, one of the major bioactive components of *S. medusa*, naturally occurs in *Bardanae fructus*, *Arctium lappa* L., *Fructus Arctii*, *Torreya nucifera* and *Ipomea cairica*. It has been reported to exhibit antioxidant, antitumor and anti-inflammatory activities [11–14] and pharmacological properties such as effect on the induction of apoptosis and the putative pathways of its action in HL-60 and K562 cells [15]. Arctigenin also exhibited potent inhibitory effects on the production of NO and the release of TNF-α and IL-6 in LPS-activated macrophages RAW 264.7 and THP-1 [16].

Traditional extraction techniques include ultrasound-assisted extraction (UAE), heat reflux extraction (HRE) and Soxhlet extraction (SE). Microwave-assisted extraction (MAE) technique was developed for the fast extraction in recent years. Many reports have been published on the application of MAE of secondary metabolites from plants [17–25]. However, MAE of arctigenin from *S. medusa* has not been reported. The objective of our study was to aim at obtaining the optimal procedure by dynamic microwave-assisted device for the extraction of arctigenin from *S. medusa*. The main advantages of dynamic microwave-assisted extraction (DMAE)

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were the considerable reduction in time and obtaining high yield of secondary metabolites from plants as compared to conventional techniques. In the paper the feasibility of DMAE was reported for the rapid extraction of arctigenin from *S. medusa* and DMAE was compared with traditional techniques.

Experimental

Plant Material, Reagents and Standard

The whole plant of *S. medusa* was collected from Qilian Mountains of Qinghai province in China, and was identified by Lijuan Mei (Northwest Plateau of Biology Institute, CAS). The whole plant of *S. medusa* was dried constantly at 60 °C and then pulverized to powder (about 20-mesh) with a disintegrator. Methanol used for high performance liquid chromatography (LC) was of chromatographic grade (Yuwang Chemical Factory, Shandong, China), and the water was ultrapure water (18.25 MΩ/cm^2). All organic solvents used for preparation of crude extract were of analytical grade (Baishi Chemical Factory, Tianjin, China). Standard of arctigenin (>98%) was obtained from chromatographic separation.

Methods

The Single Factor Test

In this experiment, each extraction was repeated three times. Four extraction methods were investigated, DMAE was performed on microwave apparatus (MG08S-2B microwave instrument, Nanjing Huiyan Microwave System Engineering Co., Nanjing, China) using reflux system with ice water and 1.0 g powder of *S. medusa* was added to 80 mL methanol and extracted for 10 min with a magnetic stirrer. UAE was conducted in an ultrasonic bath (KQ-250DB ultrasonic instrument, Kunshan Ultrasonic Instrument Co., Kunshan, China). The powder of *S. medusa* weighing 1.0 g was placed into a 500 mL glass flask with 80 mL methanol and sonicated in a water bath for 40 min. HRE (HH-6 thermostatic waterbath, Changzhou Guohua Electric Appliance Co., Changzhou, China) and SE (HH-6 thermostatic waterbath, Changzhou Guohua Electric Appliance Co., Changzhou, China) were processed in a water bath at 80 °C and 1.0 g powder of *S. medusa* was placed into two 500 mL glass flasks and extracted for 1 h with 80 and 120 mL methanol, respectively. All of the collected solvent was evaporated to dryness using rotary evaportor (N-1000D rotary evaporation, Shanghai Huixi Precision Instrument Co., Shanghai, China) at 50 °C and dissolved in a 50 mL volumetric flask with methanol, and then filtered with 0.45 μm membrane filter for LC analysis. With the best extraction method DMAE, different extraction solvents, methanol concentration, ratio of solvent to raw material, extraction time, microwave power and extraction cycles were researched in order.

Optimization of Arctigenin with DMAE

Orthogonal L_9 $(3)^4$ test design in the extraction mode was used for optimization extraction conditions. In this study, nine extractions were carried out at extraction times of 10, 15 and 20 min, ratio of liquid to raw material 1:40, 1:50 and 1:60, extraction power 125, 390 and 750 W, extraction cycles 1, 2 and 3. All levels were selected on the basis of the single-factor test. Every extraction was repeated three times. All of the collected solvent was evaporated to dryness using a rotary evaporator at 50 °C and dissolved in a 50 mL volumetric flask with methanol, then filtered with 0.45 μm membrane filter for LC analysis.

LC Analysis

Agilent 1200 system consisted of four G1311A pumps; G1316A column temperature box; G1329A auto-sampler; G1315A detector. Agilent 1200 technologies chemstation software was used. The samples and standard of arctigenin were analyzed by LC. Analysis was accomplished with Eclipse XDB-C18 column (150 mm × 4.6 mm i.d., 5 μm) with a column temperature of 25 °C. The mobile phase was methanol–water (50:50, *v*/*v*) and eluted at a constant flow rate of 1.0 mL min^{-1}. The effluents were monitored at 210 nm by a photodiode array detector. All samples were injected at least in triplicates into the LC for analyses. The standard of arctigenin was gained from chromatographic separation and structure identified by single x-ray diffraction. Area normalization method was adopted to determine the purity of arctigenin. As shown in Fig. 1a, the LC chromatogram of arctigenin revealed that the purity of arctigenin was 98.761%. The LC chromatogram of *S.medusa* shown in Fig. 1b.

Results and Discussion

Effect of Extraction Methods and Extraction Solvents

The results showed that different extraction methods had different extraction efficiency. The content of arctigenin was 8.830 ± 0.008 mg g^{-1} by DMAE, 8.523 ± 0.012 mg g^{-1} by UAE, 8.304 ± 0.014 mg g^{-1} by HRE and 8.378 ± 0.011 mg g^{-1} by SE, respectively. Extraction efficiency by DMAE with a magnetic stirrer and static microwave-assisted extraction (SMAE) without a magnetic stirrer was also compared. The extraction efficiency of arctigenin was higher 1.520 ± 0.016 mg g^{-1} by DMAE 8.830 ± 0.008 mg g^{-1} than by SAME 7.310 ± 0.005 mg g^{-1}. With DMAE, the solvents were petroleum ether, chloroform, methanol, ethanol, acetone. The results indicated that the content of arctigenin was 1.705 ± 0.005 mg g^{-1} when extracted by petroleum ether, whilst the content of arctigenin reached the highest value of 8.830 ± 0.008 mg g^{-1} when pure methanol was employed as extraction solvent. Content of arctigenin was 7.422 ± 0.003, 7.973 ± 0.011 and 8.412 ± 0.015 mg g^{-1} when extracted by chloroform, acetone and ethanol, respectively. Thus, methanol was the most efficient solvent for the extraction of arctigenin from *S. medusa*.

Effect of Aqueous Methanol Concentration

The *S. medusa* powder (1.0 g) was extracted with 80 mL aqueous methanol by DMAE at 125 W for a 10 min cycle. The concentration of aqueous methanol varied from 10 to 100% (*v*/*v*). The result showed that the content of arctigenin was greatly influenced by the aqueous methanol concentration. Content of arctigenin was 5.209 ± 0.008, 5.401 ± 0.012, 6.034 ± 0.010, 6.430 ± 0.006, 6.721 ± 0.006, 7.309 ± 0.014, 7.950 ± 0.007, 8.026 ± 0.009, 8.088 ± 0.014 and 8.252 ± 0.012 mg g^{-1} with 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% aqueous methanol, respectively. Content of arctigenin increased significantly with the increase of methanol concentration. Therefore, 100% pure methanol was applied in the following experiments.

Effect of Solvent to Material Ratio

In order to investigate the influence of solvent to material ratio on content of arctigenin, the powder of *S. medusa* (1.0 g) was extracted with methanol by DMAE at 125 W for a 10 min cycle at different ratios of solvent to material (20, 30, 40, 50, 60, 70, 80, 90, 100 mL g^{-1}, respectively). The results indicated that the content of arctigenin increased with the increase of solvent to material ratio and reached its maximum 8.790 ± 0.005 mg g^{-1} at 50 mL g^{-1}. Content of arctigenin was 7.873 ± 0.009, 7.971 ± 0.007, 8.507 ± 0.010, 8.790 ± 0.005, 8.564 ± 0.004, 8.494 ± 0.015, 8.471 ± 0.020, 8.515 ± 0.003 and 8.502 ± 0.012 mg g^{-1} with solvent to material ratio 20, 30, 40, 50, 60, 70, 80, 90 and 100 mL g^{-1}, respectively. Therefore, 50 mL g^{-1} were considered as the optimal ratio of solvent to raw material for the DMAE process.

Effect of Extraction Time

The powder of *S. medusa* (1.0 g) was extracted with 50 mL methanol by DMAE at 125 W for 5, 10, 15, 20, 25, 30, 35 and 40 min, respectively. The result showed that the content of arctigenin at the beginning increased with the increase of duration of microwave radiation and reached its maximum 8.637 ± 0.008 mg g^{-1} at 20 min. Content of arctigenin was 8.015 ± 0.004, 8.540 ± 0.006, 8.560 ± 0.003, 8.637 ± 0.008, 8.524 ± 0.017, 8.455 ± 0.028, 8.499 ± 0.019 and 8.490 ± 0.015 mg g^{-1} with extraction times of 5, 10, 15, 20, 25, 30, 35 and 40 min, respectively. Therefore, the best microwave radiation time was 20 min and was chosen as optimal for DMAE.

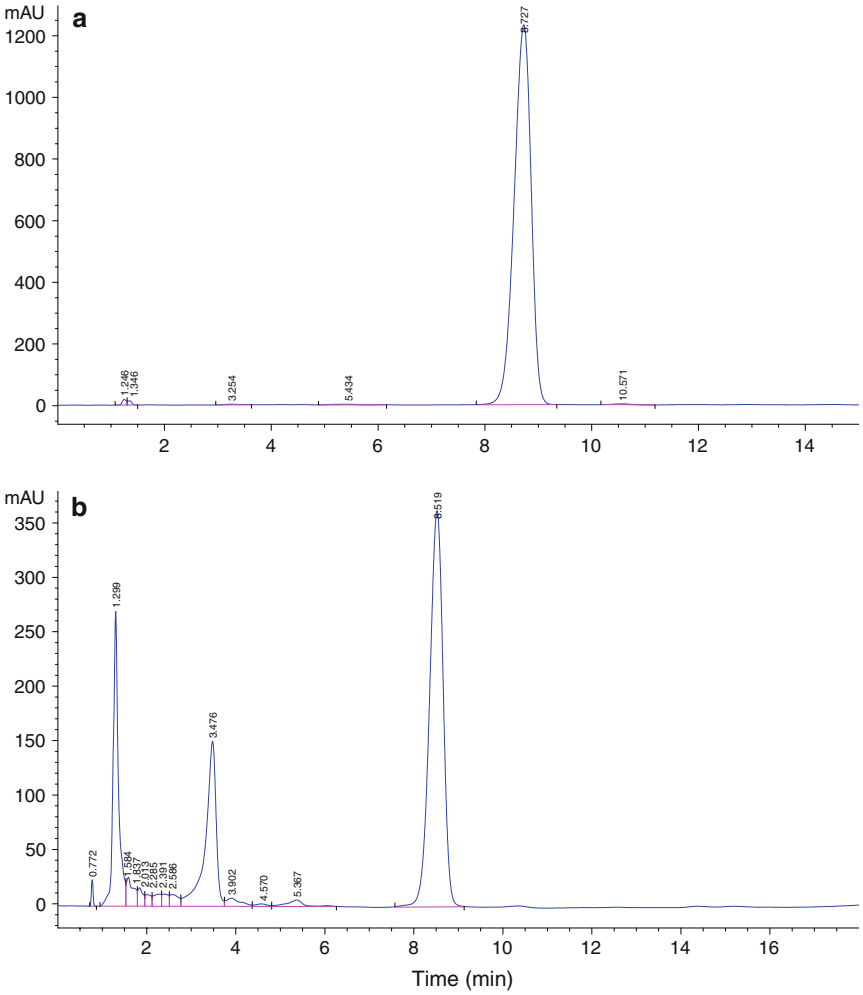

Fig. 1. LC chromatograms of arctigenin (**a**) and LC chromatogram 1.0 g powder of *S. medusa* by DMAE with 50 mL methanol for 20 min (**b**). Column: Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm); mobile phase: water (eluent A) and methanol (eluent B) (50:50). Flow rate: 1.0 mL min^{-1}; Column temperature: 25 °C; UV detection: 210 nm

Effect of Microwave Power

The powder of *S. medusa* (1.0 g) was extracted once with 50 mL methanol by DMAE for 20 min under different microwave power (60, 125, 190, 255, 325, 390, 455, 530, 610, 690, 750 W). The results indicated that the extraction efficiency had a regular increase when microwave power varied from 60 W 7.668 ± 0.012 mg g^{-1}, 125 W 7.830 ± 0.004 mg g^{-1}, 190 W 8.067 ± 0.010 mg g^{-1}, 255 W 8.106 ± 0.008 mg g^{-1}, 325 W 8.383 ± 0.013 mg g^{-1} to 390 W 8.978 ± 0.006 mg g^{-1}. But the extraction efficiency reached its equilibrium

Table 1. Analysis of $L_9(3)^4$ test results

No.	A, extraction time (min)	B, ratio of solvent to material	C, microwave power (W)	D, extraction cycles	Content of arctigenin (mg g^{-1})
1	20	50	125	3	9.567
2	20	60	390	1	9.454
3	15	40	390	3	9.186
4	15	60	125	2	7.498
5	15	50	690	1	9.729
6	10	60	690	3	8.769
7	10	40	125	1	9.116
8	20	40	690	2	9.760
9	10	50	390	2	9.649
K_1	9.178	9.354	8.727	9.433	
K_2	8.804	9.648	9.430	8.969	
K_3	9.594	8.574	9.419	9.174	
R	0.79	1.074	0.703	0.464	

R refers to the result of extreme analysis

when the microwave power varied from 390 W 8.978 ± 0.006 mg g^{-1}, 455 W 8.949 ± 0.008 mg g^{-1}, 530 W 8.931 ± 0.015 mg g^{-1}, 610 W 8.917 ± 0.024 mg g^{-1}, 690 W 8.908 ± 0.026 mg g^{-1}, 750 W 8.931 ± 0.016 mg g^{-1}. Therefore, 390 W was chosen as the optimal microwave power for DMAE.

Effect of Extraction Cycles

As to extraction cycles, the powder of *S. medusa* (1.0 g) was extracted with 100% methanol at 390 W for 20 min under solvent to material ratio 50 mL g^{-1}. The residue was taken back and re-extracted 1, 2, 3, 4 and 5 times using fresh methanol each time under the above-mentioned conditions. Content of arctigenin 8.883 ± 0.018, 9.041 ± 0.010, 8.980 ± 0.015, 8.981 ± 0.022, 8.960 ± 0.025 mg g^{-1} with 1, 2, 3, 4 and 5 extraction times. The highest content was 9.041 ± 0.010 mg g^{-1} with two cycles. Therefore, two cycles were considered as optimal extraction cycles for DMAE.

Effect of the Optimal Single Factor Experiment

From the single factor experiment, we obtained the optimal single factor extraction conditions (extraction method: DMAE; extraction solvent: methanol; ratio of solvent to raw material: 50 mL g^{-1}; extraction time: 20 min; microwave power: 125 W; extraction cycles: 2). The content of arctigenin reached 9.170 ± 0.011 mg g^{-1} on the optimal single factor extraction conditions.

Optimization of Arctigenin with DMAE

Optimization of the suitable extraction conditions in the arctigenin extraction can be carried out by using an orthogonal L_9 $(3)^4$ test design based on the results of the single factor experiment. The total evaluation index was used for analysis by a statistical method. The results of orthogonal test and extreme difference analysis are presented in Table 1. The analysis of variance was performed by statistical software SPSS 13.0. The results indicated that the maximum content of arctigenin was 9.760 ± 0.007 mg g^{-1}. However, we cannot select the best extraction conditions based only on these outcomes in Table 1, and a further orthogonal analysis was warranted. Thus, the K_1, K_2, K_3 and R values were calculated and listed in Table 1. We found that the influence to the mean content of arctigenin of the compounds decreased in the order: B > A > C > D according to the R values. The ratio of solvent to raw material was found to be the most important determinant of extraction efficiency. In other words, the maximum content of arctigenin was obtained when extraction time, ratio of solvent to raw material, microwave power and extraction cycles were 20 min, 50 mL g^{-1}, 390 W and once, respectively.

Optimization of Chromatographic Conditions in LC

Scanning from 190 to 400 and 210 nm was selected as detection wavelength for acquiring chromatograms. In order to achieve better chromatographic separation, various linear gradients of methanol–water were investigated at a flow-rate of 1.0 mL min^{-1}. Finally, methanol–water 50:50 (v/v) was chosen as it allowed the arctigenin's peak to be clearly separated with a column temperature of 25 °C. Typical chromatograms of standard and samples were shown in Fig. 1a, b.

Conclusions

An optimized DMAE process has been developed for fast extraction of arctigenin from *S. medusa*. Under the optimal extraction condition (extraction method: DMAE, extraction solvent: methanol, ratio of solvent to raw material: 50 mL g^{-1}, extraction time: 20 min, microwave power: 390 W; extraction cycle: 1), the maximum content of arctignenin reached 10.891 ± 0.003 mg g^{-1}. Compared to traditional methods, such as HRE, UAE and SE, the DMAE process shortened extraction time and improved yield of arctigenin. The DAME showed great potential for active components of extraction from other plant in industrial application in the future.

Acknowledgments

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