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Full Length Research Paper

Microwave-assisted extraction of tannins from Chinese herb *Agrimonia pilosa* Ledeb

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Tannins are water soluble polyphenols. Previous studies showed that *Agrimonia pilosa* Ledeb contains high levels of tannins. In this study, a microwave-assisted extraction (MAE) method was evaluated for the extraction of tannins. The optimal extraction conditions were as follows: irradiation power 500 W, ratio of solvent to material 35 ml/g, irradiation temperature 30°C and irradiation time 15 min. Under these conditions, the extraction yields of tannins from *A. pilosa* ledeb achieved was 128.65 mg/g. The MAE method provided a good alternative for the extraction of tannins from *A. pilosa* ledeb.

Key words: *Agrimonia pilosa* Ledeb, microwave-assisted extraction, tannins.

INTRODUCTION

Agrimonia pilosa Ledeb (APL) is a traditional medicinal plant belongs to Rosaceae in Asian countries and has been reported to possess various medicinal importance (Zhu et al., 2009). It is a famous medicinal plant used for cancer therapy in Japan and the use of its root extract has been wildly reported. In China, this plant is traditionally used to suppress diarrhoea, reduce gastric ulcers, relieve inflammation, improve eyesight, detoxify poison and increase the flow of urine (Xu et al., 2005). Nowadays, *A. pilosa* Ledeb is routinely applied on the treatment of diseases such as stomatitis, hepatitis, enteritis, hematischesis, nephritis, etc. that are caused by bacteria and virus infection. Among the polyphenolic compounds in *A. pilosa* Ledeb, tannins are the main substances with better pharmacological activities including antiviral, hepatoprotective, and antihypertensive activities (Cheng et al., 1995).

A very important chemical compound tannins found in *A. pilosa* Ledeb plant is composed of diverse group of polyphenolics which are found in higher plants including oligomers and polymers that have high molecular weight plants used as foods and feed (Haslam et al., 1989). Tannins ingested with the diet by humans or animals may affect protein utilization by forming insoluble complexes with protein, iron utilization by complexing with iron, and biological antioxidant status by participating in redox reactions (Hagerman et al., 1998). Tannins may contribute to the chemical defenses that minimize damage to plants by insect and mammalian herbivores (Felton et al., 1997). Many tannins molecules have also been shown to reduce the mutagenic effect of a number of carcinogens and mutagens. These carcinogens and mutagens produce oxygen-free radicals for interaction with cellular macromolecules. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds. The antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver

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necrosis, and modulate immunoreaction. The dosage and kind of tannins are critical to these effects.

Microwave-assisted extraction (MAE), which combines microwave and traditional solvent extraction, has gained wide acceptance as a powerful tool for sample preparation of solid matrices. In contrast with traditional extraction techniques, it possesses more advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost and lower decomposing of the target species (Proestos et al., 2007). The aims of the present work was to evaluate the feasibility of microwave-assisted extraction for extraction of tannins from *A. pilosa* Ledeb (TAP).The operational parameters were optimised using single factor experiments and orthogonal experiment design. The objective of the work is to establish the optimised condition of MAE for development and application of the Chinese herb resource.

MATERIALS AND METHODS

Plant material

A. pilosa Ledeb were collected in May from the cap mountain of Harbin District, China, and was authenticated by Prof Jin zhe-xiong from College of Pharmacy, Harbin University of Commerce. Voucher specimens were deposited in the herbarium of this Laboratory. Stems and roots were air dried and then pulverized into a homogeneous size by a disintegrator and then sieved (30 - 40 mesh).

Microwaves-assisted extraction

The microwave extractor (UWave-1000), which includes a time controller and a temperature controller, was manufactured by Shanghai Sineo microwave Products Company. In the MAE experiments, a 35 ml acetone solution was added to 1 g of dried sample powder placed in an inner vessel. A magnetic stirrer bar was placed into the vessel for thorough mixing of the solvent with the sample. The extraction was carried out with different extraction conditions. The microwave vessels were sealed and irradiated at a certain temperature (25 - 45℃) and irradiation power (300 - 700W). After microwave irradiation, the vessels were allowed to cool to room temperature. The extracts were centrifuged at room temperature for 10 min at 10000 rpm. The supernatants were filtered through a 0.45 μm nylon membrane followed by a two fold dilution with demonized water. There were three replications of each treatment (Yang et al., 2010).

Determination of tannins

The TU-1810 spectrophotometer (Purkinje General, China) was used to determine the content of tannins in the above isolated product at 275 nm (Ribereau-Gayon et al., 2000). The tannins content was calculated using the following linear equation based on the calibration curve prepared by gallic acid, ranging from 1 to 10 $\mu g ml^{-1}$

$$A = 0.1196C + 0.0398, R^2=0.9996.$$

Where; A is the absorbance, C is the tannins content in $\mu g\ ml^{-1}$.

RESULTS AND DISCUSSION

Effect of solvent volume on extraction yield of TAP

In investigating the influence of ratio of solvent to material on yields of tannins from *A. pilosa* Ledeb, several tests were performed at different ratios of solvent to material. The rest of the variables employed were extraction temperature 30℃, extraction time 15 min and microwave irradiation power 500 W. It can be seen in Figure 1 that the yields of tannins increased with increasing ratios of solvent to material during the range 25 – 35 ml/g. But, in the range of 35 - 50 ml/g, there was no significant difference or enhancement, which was probably due to the larger volume of acetone solution causing excessive swelling of the materials by water and absorbing the effective constituent (Guo et al., 2001) or that a higher acetone concentration, proteins could be coagulated and some lipid compounds may be extracted, which can make larger diffusion resistance and, thus, limit the extraction content. Hence, a value of 35 ml/g was considered as the optimal ratio of solvent to material for the MAE process.

Effect of microwave power on the extraction yield of TAP

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipolerotation 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 solvent and matrix, allowing the dissolution of components to be extracted (Duan et al., 2001; Hu, 2003; Guo et al., 2003). 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 and 700 W. As shown in Figure 2, the high microwave irradiation power enhanced the yields of TAP when the power was lower than 500 W. However, when the power was higher than 500 W, the yields declined. This may be due to different materials having different appropriate microwave irradiation power. High irradiation power can offer superfluous energy to the solvent and matrix. It can slightly disorder molecular interactions. The abnormal molecular interaction structure can affect the extraction yields of TAP. Hence, 500 W was chosen as the appropriate microwave irradiation power.

Effect of extraction time on the extraction yield of TAP

It is necessary to select a proper irradiation time to

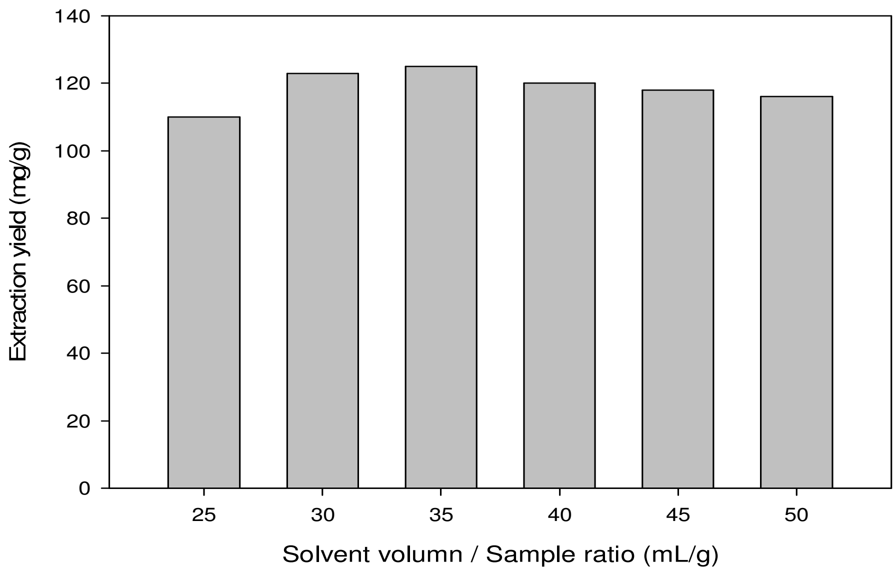

Figure 1. Effect of solvent volume on the extraction yield of TAP.

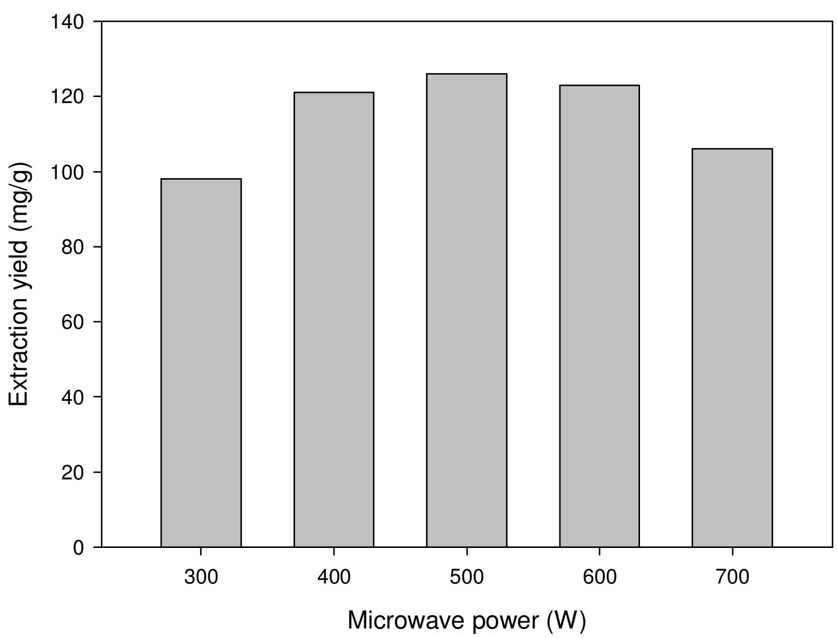

Figure 2. Effect of microwave power on the extraction yield of TAP.

guarantee completion of the extraction. Studies were carried out at different times, e.g., 5, 10, 15, 20, 25 and 30 min. As depicted in Figure 3, with increasing irradiation times from1 to 15 min, the extraction yields of TAP increased rapidly and reached its maximum at 15 min. Then, the extraction yields decreased with the extension of the irradiation time. This may be due to the decomposition of TAP at long irradiation time. Thus, 15 min was considered as the appropriate irradiation time.

Effect of extraction temperature on the extraction yield of TAP

Extraction temperature is a factor that must be studied to increase the effectiveness of extraction of analytes

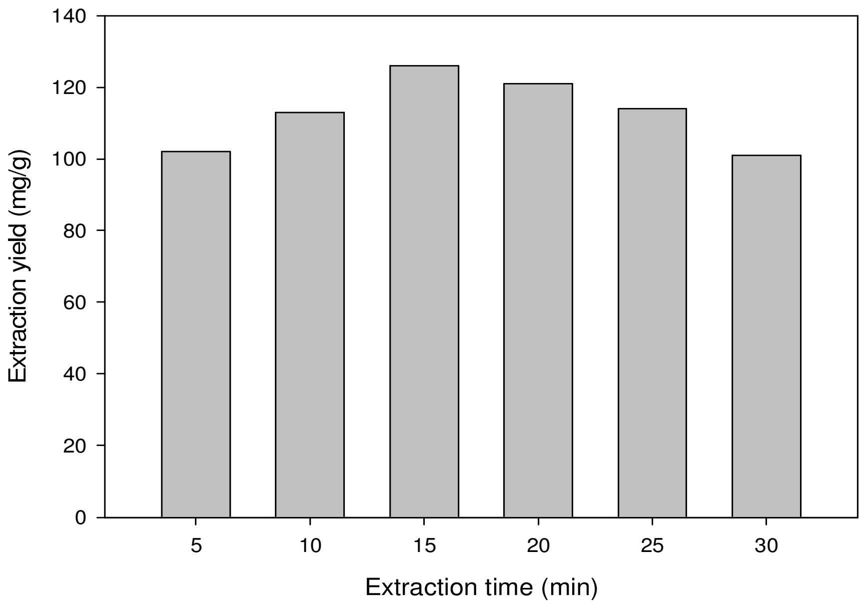

Figure 3. Effect of extraction time on the extraction yield of TAP.

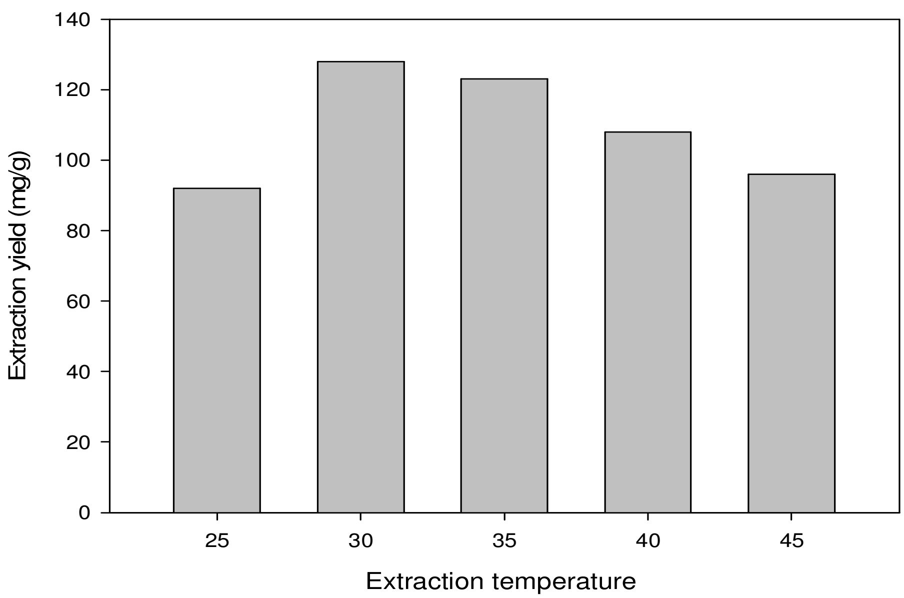

Figure 4. Effect of extraction temperature (℃) on the extraction yield of TAP

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 TAP during MAE, an amount of 5.0 g materials were extracted for 15 min and ratio of solvent to material 35 g/ml under 500 W microwave irradiation power at different temperatures, repeated in three cycles. Figure 4 shows that the yields of TAP increased remarkably with increasing temperatures from 25 to 30℃. Above 30℃, the yields of TAP changed slowly and even decreased. Increasing moderate temperatures may also cause an opening of the cell matrix, and as a result, improve extraction of TAP. But, exceeded moderate temperature, solvent viscosity decreased and the diffusivity increased, hence, the efficiency of extraction decreased (Camel et al., 2000; Pan et al., 2000). Then, 30℃ was selected as the optimum temperature for extraction.

Table 1. The factors and levels for the orthogonal design.

Level	Solvent volume	Microwave power	Extraction time	Extraction temperature
	A	B	C	D
1	30	300	10	25
2	35	400	15	30
3	40	500	20	35

Table 2. The results of orthogonal experiment L9 (3^4).

Test	A	B	C	D	Extraction yield
1	1	1	1	1	118.21
2	1	2	2	2	126.25
3	1	3	3	3	119.45
4	2	1	2	3	122.68
5	2	2	3	1	124.35
6	2	3	1	2	123.24
7	3	1	3	2	121.67
8	3	2	1	3	108.56
9	3	3	2	1	120.48
K1	121.30	120.85	116.67	121.01	
K2	123.42	119.72	123.14	123.72	
K3	116.90	120.06	121.82	116.90	
R	6.52	1.34	6.47	6.82	

Optimization of microwave-assisted extraction conditions of TAP

The optimization technique is a unique and powerful optimization discipline that allows optimization with minimum number of experiments (Yang et al., 2008). Thus by MAE method, it is possible to reduce the time and cost for experimental investigations and improve the performance characteristics. Since different factors affect the MAE process, the optimization of the experimental conditions represents a critical step in the development of a MAE method (Pan et al., 2008). In the present study, three levels are defined for each of the factors as summarized in Table 1.

A L9 orthogonal array scheme was adapted which needs 9 experiments to complete the optimization process. The extraction results performed under orthogonal design conditions are shown in Table 2.The sequence in which the experiments were carried out was randomized to avoid any kind of personal or subjective bias. As seen from Table 2, we can find that the influence to the mean extraction yields of decreases in the order: D > A > C > B according to the R values. Microwave power was found to be the most important determinant of extraction yield of TAP. The best combination shown was A2B3C2D2 which is in specific, irradiation temperature was 30℃, solvent volume was 35 ml, microwave power was 500 W and extraction time was 15 min. That was also the optimal extraction condition, while extraction yield of was 128 mg/g.

Conclusion

The results presented in this study demonstrated the feasibility of MAE for tannins from *A. pilosa* Ledeb. The optimal condition through single factor experiments and orthogonal experiment was determined as followings: Microwave irradiation power 500 W, ratio of solvent to material 35 ml/g, irradiation temperature 30℃, extraction time 15 min. At optimised conditions of MAE in the present study, the extraction yield of tannins was 128.65 mg/g, which represented an increase of 46.01%, as compared with the conventional method (Wei et al., 2007; Yu et al., 2010). Our results show that MAE is an alternative method for the natural products extraction from plants because of its higher efficiency and because it can reduce experimental costs.

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