Research Article

Analysis of Rutin from *Lespedeza virgata* (Thunb.) DC. by Microwave-Assisted Extraction and Capillary Electrophoresis

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A simple and rapid microwave-assisted extraction (MAE) technique was developed for the extraction of rutin from *Lespedeza virgata* (Thunb.) DC. The influence of four extraction variables on extraction of rutin was discussed. The optimum extraction conditions found were 75% aqueous ethanol, solvent volume to sample weight ratio at 90 : 1, extracting temperature at 75°C, and extraction time for 15 min. A comparison was made among MAE, classical maceration, and ultrasonic-assisted extraction (UAE). Yields were determined by high-performance capillary electrophoresis (HPCE). The whole analysis process was completed in ten minutes. The needful volumes of sample and buffer are very little. Compared with maceration and ultrasonic extraction, MAE is a rapid method with higher yield and less solvent consumption.

1. Introduction

Lespedeza virgata (Thunb.) DC. is a plant which has been used as Chinese folk remedies for thousands of years. Traditional Chinese medicines (TCMs) are used in clinical therapy of many diseases. Recently, due to their high pharmacological activity and low toxicity, TCMs have been increasing researchers' interests worldwide for the prevention and treatment of various illnesses. The most important bioactive compounds presented within *Lespedeza virgata* (Thunb.) DC. are flavones including rutin and quercetin. These flavones have shown significant scavenging of free oxygen radicals and antidecrepitude activity [1–3]. Therefore, an effective and rapid extraction of flavones from *Lespedeza virgata* (Thunb.) DC. is founded with considerable significance.

Microwave heating has already been widely applied in solvent extraction because of its main advantages like rapidity and high efficiency. It has been already proved in many cases that microwave-assisted extraction (MAE) is a viable alternative to conventional technique for many kinds of samples [4–6]. Although most investigations were devoted to determine the organic contaminants such as PAHs [7] and PCBs [8] in environmental samples, recently MAE technique has also started to find its way into biologically active compounds, such as the extraction of artemisinin from

annual, the extraction of puerarin from *Radix puerariae* [9–11].

In this study, MAE of rutin from *Lespedeza virgata* (Thunb.) DC. was presented and evaluated by comparing with the conventional techniques. It was reported [12] that the use of microwave transparent solvents would induce a sudden temperature increase inside cellular structures, since the water in the cells would heat, which may result in an eventual rupturing of the cell walls and the rapid release of their constituents into the surrounding medium. The high yields of rutin extracted by MAE could be explained by the phenomena that the destruction of the plant structure occurred during the extraction process.

2. Experimental

2.1. Plant Materials and Chemicals. Standard rutin was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. *Lespedeza virgata* (Thunb.) DC. was collected in Hubei Province, China. Analytical grade ethanol (Beijing Chemical Reagent Factory, China) was used. Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

The leaves of *Lespedeza virgata* (Thunb.) DC. were dried in vacuum at 60°C for 24 h, crumbled, and then sieved with a

140 mesh screen. Twig powders with particle size under 140 meshes were used as the practical samples.

Stock solution of rutin (2.50 mg/mL) was prepared in ethanol. One milliliter of each stock solution was transferred into a 10 mL volumetric flask and diluted with buffer to obtain standard mixed solutions. These solutions were then diluted with buffer stepwise to obtain a series of working solutions. All stock solutions and working solutions were stored at 4 °C in a refrigerator and brought to room temperature before use.

2.2. Microwave-Assisted Extraction (MAE). MAE experiments were performed with an XH-100A microwave synthesis/extraction system (maximal power: 1000 W, Beijing XiangHu Science and Technology Development Company, Beijing, China). The XH-100A instrument has an internal temperature control system with a temperature probe system, which monitors the temperature inside the flask. Add 80 mL ethanol solution to 1.0 g of dried leaves placed in a flask. The samples dealt with different extraction conditions. Extraction solutions were filtered through a 0.22 μ m filter prior to electrophoresis analysis.

2.3. Maceration. Maceration was performed with 1.0 g of dried leaves and 100 mL ethanol solution. The mixture was left at ambient temperature for 6 h, with sporadic shaking with a glass agitator. After extraction, solvent was collected to concentrate by vacuum rotator evaporation with a temperature controlled bath and filtered through a 0.22 μ m filter prior to electrophoresis analysis.

2.4. Ultrasound-Assisted Extraction (UAE). Ultrasound-assisted extraction (UAE) was carried out in an ultrasonic bath (power: 100 W). One gram of dried leaves was placed in a beaker with 80 mL of 75% ethanol solution. The beaker was immersed in the ultrasonic bath and the extracting procedure lasted for 90 min. The sample was filtered through a 0.22 μ m filter prior to electrophoresis analysis.

2.5. Capillary Electrophoresis (CE). The CE was performed using a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) under a normal polarity separation mode. A capillary (Beckman Coulter, Fullerton, CA, USA) with 30 cm effective length (40 cm total) and an inner diameter (I.D.) of 75 μ m were used with an applied voltage of 10 kV at 25 °C. The capillary was rinsed successively with 0.01 mol/L NaOH for 30 min, deionized water for 30 min, and running buffer for 20 min when it was first used. The temperature of the separation capillary column was thermostated at 25 °C.

Among each run, the capillary was rinsed with 0.01 mol/L NaOH for 5 min and then with the electrophoresis buffer for 3 min. The separation buffer was 6.25 mM $\text{Na}_2\text{B}_4\text{O}_7$ solution (pH 9.0), and the applied voltage was 10 kV. The electrophoresis buffers were filtered through 0.22 μ m filter before use. The mixed standard samples or extraction samples were dissolved in 10 mL of methanol and filtered (0.22 μ m (Millipore)) for CE analysis. Samples were injected into the

FIGURE 1: Effect of the concentration of ethanol solution on rutin extraction yield from *Lespedeza virgata* (Thunb.) DC.

capillary using pressure at 0.5 psi for 3 s. The absorbance was measured at a wavelength of 254 nm for the detection of rutin.

3. Results and Discussion

3.1. Optimization of Microwave-Assisted Extraction (MAE). For MAE, a number of extraction parameters such as the concentration of ethanol solution, ratio of solution to solid (mL/g), extracting temperature, and extraction time were investigated.

3.1.1. Effect of the Concentration of Ethanol Solution. The selection of an appropriate ethanol concentration as an extracting solvent is crucial. Different concentrations of ethanol solutions were tested under the same condition using the same protocol. The results were summarized in Figure 1. As shown, the suitable concentration was 75% ethanol at which the maximal rutin extraction yield was found.

3.1.2. Effect of Ratio of Solution to Solid. The ratio of solution to solid was optimized to increase the extraction efficiency and decrease the solvent consumption. Experiments were performed by increasing the ratio from 50 to 90 under the experimental conditions described previously. From the results shown in Figure 2, a ratio of 80 was evidently considered to be enough for extraction.

3.1.3. Effect of Extraction Temperature. Heating is useful to increase the extraction yield. However, heating too long might cause thermal decomposition of some bioactive compounds at high temperature [13]. This result was confirmed by our test at ranges of temperatures between 70 and 90 °C, which was not found that the higher temperature, the greater extraction yields. Extractions at different extraction temperature, such as at 70, 75, 80, 85, and 90 °C for 15 min, were tested. Results were shown in Figure 3. From these results, a temperature of 75 °C was chosen for further extraction experiments.

FIGURE 2: Effect of the ratio of solution to solid on rutin extraction yield from *Lespedeza virgata* (Thunb.) DC.

3.1.4. Effect of Extraction Time. Extractions were investigated during irradiation times of 5, 10, 15, 20, 25 min, and the extraction efficiency was shown in Figure 4. In the first five minutes, the solvent mixture absorbed the microwave energy to chiefly raise the temperature for itself. This explained the lower yields obtained during the earlier extraction time. Rapid extraction occurs at 15 min. According to Figure 4, the efficiencies are lower after 15 min, suggesting that the target is maybe decomposed.

3.1.5. Orthogonal Experiment Design and Analytical Result. Orthogonal experimental design is an optimization method for researching multiple factors and levels [14]. It utilizes orthogonal table to arrange the experiment scientifically and evaluate multiple factors. According to the orthogonality, some representative tests can be chosen from overall tests, and the result from representative tests can be used to find the optimal scheme and discover the unanticipated important information. The first step of orthogonal optimization is accomplished for screening the factors studied (concentration of ethanol solution, ratio of solvent volume to solid, extraction temperature, and microwave extraction time) in order to obtain their significant level on the partition coefficient. The orthogonal table $L_9(3^4)$ is used to arrange the experiments; four factors are evaluated each time, and each factor takes three levels.

According to Table 1, the following results can be concluded: (1) concentration of ethanol solution and extraction temperature have the largest range R among four factors, which shows they have the greatest influence, followed by ratio of solvent volume to solid and microwave extraction time; (2) from the table, it is easy to find out that the combination of group six extracts rutin much more than others, where the concentration of ethanol solution is 75%, solvent volume to sample weight ratio is 90:1, the extraction temperature is 70°C, and microwave extraction time is 15 min. I_{1j} , II_{2j} , III_{3j} are the average extracted amount of rutin at the different level of the various factors. R is range (average extraction

FIGURE 3: Effect of the extraction temperature on rutin extraction yield from *Lespedeza virgata* (Thunb.) DC.

FIGURE 4: Effect of extraction time on rutin extraction yield from *Lespedeza virgata* (Thunb.) DC.

of the maximum minus minimum). It indicates whether the changes in the level of the various factors affect the extraction amount. However, after calculating and comparing the magnitudes of I_{1j} , II_{2j} , and III_{3j} , the combination in this batch of test extracting rutin most easily must be that the concentration of ethanol solution is 75%, solvent volume to sample weight ratio is 90:1, the extraction temperature is 75°C, and microwave extraction time is 15 min.

3.2. Quantitative Analysis Method and CE Results. Following MAE, CE was used to identify rutin extracted from *Lespedeza virgata* (Thunb.) DC. The electropherograms of standard and microwave-assisted extraction sample from *Lespedeza virgata* (Thunb.) DC. were shown in Figures 5 and 6, respectively. The retention time of rutin was about 5.5 min under the optimal separation condition. The calibration curves (correlation

TABLE 1: Orthogonal experimental L_9 (3^4) and analytical result.

Factor	Concentration of ethanol solution/% (A)	Ratio of solvent volume to solid/mL·g ⁻¹ (B)	Extraction temperature/°C (C)	Microwave extraction time/min (D)	Extraction yield/mg·g ⁻¹
1	70 (1)	70 : 1 (1)	70 (1)	10 (1)	0.244
2	70 (1)	80 : 1 (2)	75 (2)	15 (2)	0.268
3	70 (1)	90 : 1 (3)	80 (3)	20 (3)	0.254
4	75 (2)	70 : 1 (1)	75 (2)	20 (3)	0.265
5	75 (2)	80 : 1 (2)	80 (3)	10 (1)	0.256
6	75 (2)	90 : 1 (3)	70 (1)	15 (2)	0.270
7	80 (3)	70 : 1 (1)	80 (3)	15 (2)	0.248
8	80 (3)	80 : 1 (2)	70 (1)	20 (3)	0.252
9	80 (3)	90 : 1 (3)	75 (2)	10 (1)	0.258
I_{1j}	0.255	0.252	0.255	0.253	—
II_{2j}	0.264	0.259	0.264	0.262	—
III_{3j}	0.253	0.261	0.253	0.257	—
R	0.011	0.009	0.011	0.009	—

$I_{1j} + II_{2j} + III_{3j} = 0.772$. I_{1j} , II_{2j} , III_{3j} are the average extracted amount of rutin at the different level of the various factors. R is range (average extraction of the maximum minus minimum).

TABLE 2: Comparison between maceration, ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE).

Comparison	Maceration	Ultrasonic-assisted extraction (UAE)	Microwave-assisted extraction (MAE)
Extraction time/(min)	360	90	15
Extractant volume/(mL)	100	80	80
Rutin extraction yield/(mg/g)	0.179 ± 0.016	0.225 ± 0.011	0.271 ± 0.012

FIGURE 5: The electropherogram of standard substances.

FIGURE 6: The electropherogram of dried leaves extract samples.

coefficient) for rutin were studied and found to be linear in the range of 10–800 mg/L. The linear equation was $A = 2 \times 10^6 c - 4034.1$ ($n = 6$, $R = 0.9999$), where A , c , and R were the concentration of the analytes (mg/L), the peak area, and the correlation coefficient, respectively. The limit of detection (LOD), defined as the lowest sample concentration which can be detected ($S/N > 3$), was 2.42 mg/L.

HPCE and HPLC can be each other's complement. Capillary electrophoresis has advantages such as separation efficiency and resolution, small amounts of solvent and sample, low cost. Compared with HPLC [15], the solvent

consumes, and sample dosages of CE are almost centesimal. The injection volume of HPLC requires microliter level, and which of CE is only nanoliter. Meanwhile the consumption of mobile phase in CE is less than that of HPLC. HPCE transporting system does not contain the pump, therefore the relative cost is reduced. According to different molecule character (if size, electric charge count, hand collects nature, the sparse ability in swimming waits), CE can carry out effective separation on extremely broad scopes. By contrast, to achieve similar purpose, HPLC needs the expensive pillar and solvent consuming a lot of price.

3.3. Comparison among Various Extraction Methods. It was demonstrated in our tests that microwaves improved much the extraction efficiencies of plant essential oil compounds [16, 17]. The extraction efficiency of MAE was compared with that of the maceration and ultrasonic extraction in the study. The obtained results were summarized in Table 2. Ultrasonic extraction and MAE gave significantly higher values, compared to maceration values. With respect to the extraction time, MAE was the shortest, requiring just 15 min. Maceration lasted 360 min, while ultrasonic extraction required 90 min. Concerning the extraction efficiency, ultrasonic extraction was found to be similar to MAE. The maceration method, however, does not fit for extracting rutin due to its much lower extraction efficiency than MAE and UAE. Evidently, MAE had a considerably shorter extraction time than UAE, though it had a limited higher extraction yield. For an extraction procedure under the same extractant volume, UAE took 90 min, while MAE took 15 min only. The energy required to perform the ultrasonic extraction is much greater than MAE. In this study, MAE is suitable for extraction of the active components.

4. Conclusions

The MAE-HPCE of rutin from *Lespedeza virgata* (Thunb.) DC. was established in this study. This experiment is completed effectively and rapidly by using orthogonal method to design experimental scheme. The experimental results showed that the concentration of ethanol solution, ratio of solvent volume to solid, the extraction temperature, and microwave extraction time all affect the rutin extraction value. We used three techniques to extract rutin from dried *Lespedeza virgata* (Thunb.) DC. They were investigated and compared. Both UAE and MAE showed high yields and low solvent consumptions. It was found that the extraction time with MAE was significantly shortened. The analysis process by HPCE was completed in ten minutes and friendly to environment. Finally, the MAE-HPCE method was shown to be very efficient and rapid in the extraction and analysis of rutin from *Lespedeza virgata* (Thunb.) DC.

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