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Optimization of microwave-assisted extraction of resveratrol from polygonum cuspidatum sieb et Zucc by orthogonal experiment

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ABSTRACT

In order to optimize the conditions for the microwave-assisted extraction of resveratrol from Polygonum cuspidatum Sieb et Zucc, in basis of microwave-assisted extraction time,solvent concentration , ratio of liquid to solid, microwave power four single factor test, the optimum conditions for the microwave-assisted extraction of resveratrol from Polygonum cuspidatum Sieb et Zucc are determined by orthogonal experiment. The optimum extraction conditions confirmed by the index of extraction yield were as follows : microwave-assisted extraction time 7 min, solvent concentration 80% (v/v)ethanol, ratio of liquid to solid 25:1 (ml:g),microwave power 1.5 kw. The extraction yield of resveratrol was 1.76%. Mass spectrum analysis showed that extract was resveratrol.

KEYWORDS

Polygonum cuspidatum sieb et Zucc;
Resveratrol;
Orthogonal experiment;
Microwave-assisted extraction.

INTRODUCTION

Polygonum cuspidatum Sieb et Zucc, a Chinese traditional herbal medicine, is used for eliminating stasis to activate blood circulation, relieving pain, expelling phlegm and arresting coughing. The main components in it is resveratrol, piceid etc[1]. Resveratrol is widely used in medicine, health products and cosmetic industries on account of its various pharmaceutical properties such as anti-inflammatory, anticancer and cardioprotective activities[2,3]. Microwave activation, an unconventional energy source, has become a very popular and useful technology in organic chemistry. Microwave-assisted extractions have gathered momentum in recent years, mainly because of their elegance, high yield, short time span, and ecofriendly conditions[4-7]. Most of the active ingredients in Chinese herbal medicines are wrapped within the plant cell wall, thus it is difficult to damage the cell wall with a general organic solvent extraction and the extraction yield is low. Studies have shown that microwave energy can loose cell walls and reduce the mass transfer resistance and accelerate the release of the active ingredient.

In this paper, microwave-assisted extraction was applied to extract resveratrol from Polygonum cuspidatum Sieb et Zucc with ethanol solution and microwave-assisted extraction conditions were optimized by orthogonal experiment.

MATERIALS AND METHODS

Chemicals and materials

Dried root of Polygonum cuspidatum Sieb et Zucc was obtained from Shengshengtang Medicine Cooperation (Liaoyang,China),ground into powder and

passed through a 100 mesh sieve. Resveratrol standard substance (purity> 98%) were purchased from Sigma Chemical Co, stored at 4 °C Other reagents are of analytical grade.

Microwave-assisted extraction

Microwave-assisted extraction method

MDS-6 auto-frequency modulation microwave with digestion/extraction oven and temperature/pressure control (Shanghai Sineo Microwave Chemistry Technology Co., Ltd. P.R.China) was used for microwave-assisted extraction. After a serials of trials, the temperature and time of oven were adjusted to a proper range suitable for Polygonum cuspidatum Sieb et Zucc. An accurately-weighed Polygonum cuspidatum Sieb et Zucc together with ethanol solution (solvent) were placed in the extraction vessel (made of polytetrafluoro ethylene), and the vessel was set on rotating pan of digestion /extraction oven. After heating for 1~8 minutes, the mixture was centrifuged at 2000 rev/min for 10 minutes. Absorbance values of the extract were measured by UV spectrophotometry. The calibration curve was drawn by absorbance values. The mass concentration was calculated by the calibration curve and the yield of resveratrol was also calculated.

Analytical method

(1) Qualitative analysis

Thin-layer chromatography was used for qualitative analysis test. Thin layer plate is silica gel plate for 20 cm × 10 cm and eluent is toluene : ethyl acetate : acetic acid (15:3:1, v:v:v), and color is ferric chloride - potassium ferricyanide solution, and Rf values of extract were compared to that of standard sample.

(2) Quantitative analysis

UV spectrophotometry was used for quantitative analysis test. Absorbance value A of the extract is directly proportional to the concentration of extract c according to Beer's law (A =μ×b×c)when the coefficient of molar absorptionμand the thickness of cuvette b is not changed. The absorbance value at the maximum absorption wavelength of active ingredient is measured with the UV spectrophotometer and the absorbance value is proportional to extracted amount. There has the maximum absorption value at 306 nm for resveratrol. The mass concentration of resveratrol was calculated by the calibration curve , thereby extract yield was also calculated when the extract was refined and UV absorbance value was measured.

Mass spectrum

ESI/MS was used to determine the molecular weight of the sample. Electrospray ionization ESI, Cone voltage 25 V, degassing temperature 340 °C, capillary voltage 3.0 kV, desolvation gas velocity 600 L/h, quality scanning range (m/z) 0 ~ 400 was detection conditions.

RESULTS AND DISCUSSION

Drawing resveratrol calibration curve and calculating extraction yield

Establishing resveratrol calibration curve equation : the standard resveratrol 5 mg was precisely weighed and was placed in a 50 ml volumetric flask. Methanol was used to dissolve the standard resveratrol in the flask and 0.10 mg/ml standard resveratrol solution was obtained.0.15, 0.25, 0.55, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25 ,2.50 and the 3.0 ml were precisely removed and placed in a 25 ml volumetric flask, then were diluted to scale with methanol respectively. 0.6, 1.4, 2.2, 3, 4, 5 ,6 ,7 ,8 ,9 ,10 ,12 g/ml resveratrol standard solution were achieved and the absorbance values at wavelength of 306 nm was measured, respectively. Clibration curve graph was obtained when the absorbance value was abscissa and concentration was ordinate. The curve equation was as follow:

$y = 0.04019 + 29.8988x$ $R^2=0.99985$

y-absorbance value x- mass concentration ¼g/ml

Determination resveratrol content: supernatant was diluted to an appropriate multiples and absorbance value was measured at the wavelength of 306 nm. Resveratrol extraction yield was calculated.

$$\text{Extraction yield (\%)} = \frac{resveratrol\ mass(g)}{polygonum\ cuspidatum\ mass(g)} \times 100\%$$

Single factor experiment and analysis

The effect of microwave time on extraction yield

4.000g Polygonum cuspidatum Sieb et Zucc were taken and 80% (v/v)ethanol solution 80ml were extractant, and the effect of microwave time on extraction yield when the extract time were 1,2,3,4,5,6,7,8 min

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respectively was shown in Figure 1.

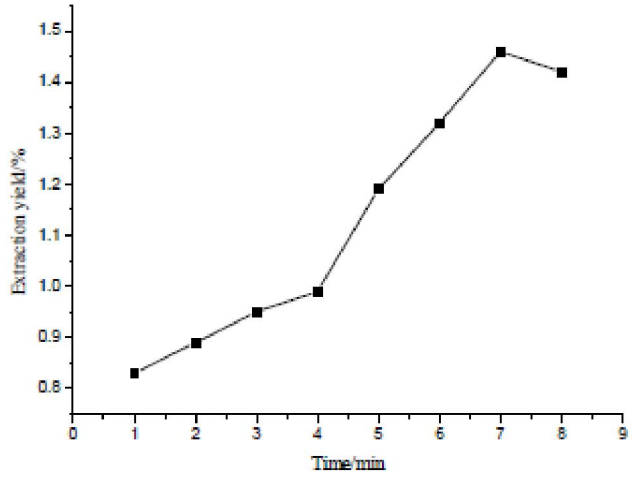

Figure 1 : The effect of microwave time on extraction yield

Figure 1 shows that extraction yields increase with increased extraction time and reach maximum when the extraction time is 7 min. Extraction yields begin to decline when microwave-assisted extraction time continues to increase. The extract may be decomposed with time increasing, which results in extraction yields reduction. Therefore, extraction time 7 min is chosen as the optimal time.

The effect of liquid -solid ratio on extraction yield

4.000g Polygonum cuspidatum Sieb et Zucc were taken and 80% (v/v) ethanol solution 80ml were extractant ,and the effect of ratio of liquid to solid on extraction yield when the extract time was 7 min was shown in Figure 2.

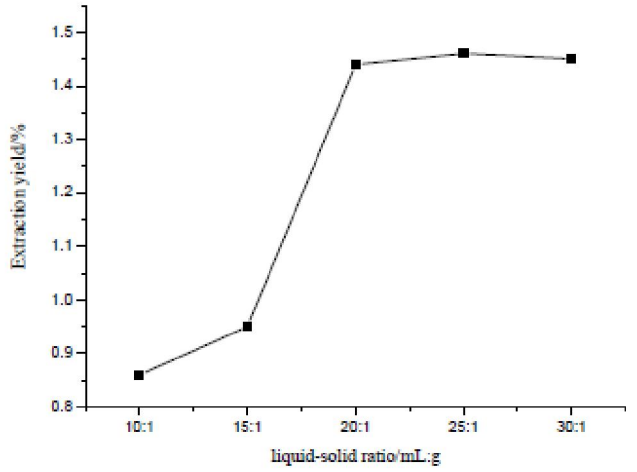

Figure 2 : The effect of liquid -solid ratio on extraction yield

We investigated the effect of liquid to solid ratio at different ratio of 10:1, 15:1, 20:1, 25:1, 30:1 (ml:g),respectively. Figure 2 shows that extraction yields increase with increased liquid to solid ratio and reach its maximum when the liquid to solid ratio is 25:1(ml:g). Hence, 25:1(ml:g) is selected as the optimum ratio of liquid to solid.

The effect of ethanol concentration on extraction yield

4.000g Polygonum cuspidatum Sieb et Zucc were taken and ratio of liquid to solid was 25:1(ml:g) ,and the effect of ethanol concentration on extraction yield when the extract time was 7 min was shown in Figure3.

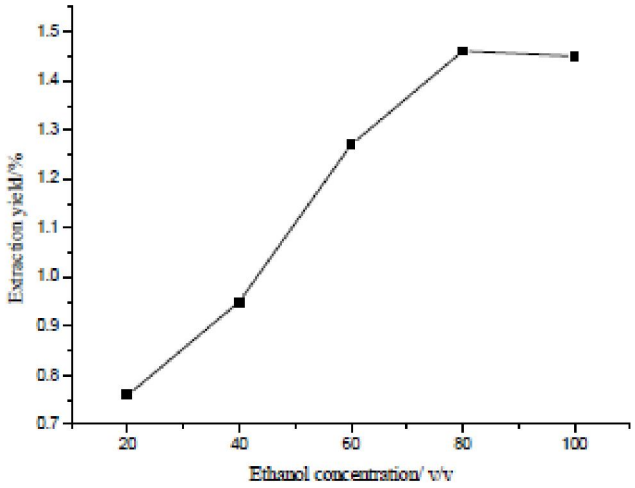

Figure 3 : The effect of ethanol concentration on extraction yield

We investigated the effect of ethanol concentration at different ratio 20%,40%,60%,80%,100%(v/v),respectively. Figure 3 shows that extraction yields increase with ethanol concentration increasing. The resveratrol extraction yields change little when ethanol concentration is more than 80% (v/v). Solubility of resveratrol in 80% (v/v) ethanol solution may reach a maximum, which results in resveratrol extraction yields few change after ethanol concentration achieved 80% (v/v).

The effect of microwave power on resveratrol extraction yield was also investigated.

Orthogonal $L_9(3^4)$test analyses

The microwave heating is different from the traditional heating method. It can penetrate the container, solvent and sample absorb directly microwave heating to achieve the effect of the hot extraction. According to single factor experiments analyses under the microwave-

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assisted extraction conditions, the main factors affecting extraction were ratio of liquid to solid , extraction time, ethanol concentration, and microwave irradiation power. Microwave-assisted extraction conditions were optimized by orthogonal experimental methods and an orthogonal $L_9(3^4)$test design was used. In this study, nine extraction tests were performed and the extraction conditions were given in TABLE 1. The results of the orthogonal test and analyses are shown in TABLE 2 and TABLE 3. Variance analysis was performed by statistical method.

TABLE 1: Factors and levels for orthogonal test

No.	A Microwave power (kw)	B microwave-assisted time(min)	C ethanol concentration (v/v)	D Ratio of liquid to solid (ml:g)
1	1.5	7	60%	25:1
2	1.0	8	80%	30:1
3	0.5	9	100%	35:1

TABLE 2 : Orthogonal L_9 (3^4)test results analysis

No.	A	B	C	D	Extraction yield(%)
1	1	1	1	1	1.63
2	1	2	2	2	1.71
3	1	3	3	3	1.58
4	2	1	2	3	1.80
5	2	2	3	1	1.55
6	2	3	1	2	1.52
7	3	1	3	2	1.39
8	3	2	1	3	1.27
9	3	3	2	1	1.57
K_1	4.92	4.81	4.42	4.74	
K_2	4.87	4.53	5.08	4.62	
K_3	4.22	4.67	4.51	4.65	
k_1	1.64	1.60	1.47	1.58	
k_2	1.62	1.51	1.69	1.54	
k_3	1.41	1.56	1.50	1.55	
R	0.23	0.09	0.22	0.04	

The extraction process was potentially affected by various factors, but microwave irradiation power, extraction time, ethanol concentration and the ratio of liquid to solid were generally considered to be the most important. Extracts were obtained from each specific test and extraction yields were calculated. The results of experiments are shown in TABLE 2. The order of A>C>B>D was determined for microwave-assisted extraction. According to TABLE 2, the optimum extraction conditions are $A_1B_1C_2D_1$ and supplementary test results are listed in TABLE 4.

TABLE 3 : Variance analysis of the orthogonal test

Variationsources	SS	Df	MS	F	P
A	0.100	2	0.050	12.45	<0.05
B	0.013	2			
C	0.086	2	0.043	10.65	<0.05
D	0.003	2			
Error(B+D)	0.016	4	0.004		

TABLE 4 : Supplementary test results

No.	A (kw)	B (min)	C (v/v)	D (ml/g)	extractionyield (%)
1	1.5	7	80%	25:1	1.76
2	2.0	7	80%	25:1	1.77
3	1.5	6	80%	25:1	1.65
4	1.5	7	80%	20:1	1.61

Compared with the other parameters, microwave irradiation power plays an important role in microwave-assisted extraction. Different microwave irradiation power were investigated from 0.5 to 1.5kw on extraction ion conditions ar GB yields, and reached its maximum at1.5kw. This is related to microwave energy penetrate materials and produce a volumetrically distributed heat source by molecular friction, resulting from dipolar rotation of polar solvents and from the conductive migration of dissolved ions. Extraction improves extraction yields when the microwave power is increased from 0.5 to 1.5 kw. However, extraction yields are not significantly increased from 1.5 to 2.0 kw. TABLE 2 and TABLE 4 also show that there is no obvious increase of extraction yields with the microwave irradiation power increasing. The higher microwave irradiation power may destroy resveratrol molecular structure, which leads to little change in extraction yields. Therefore, 1.5 kw is selected as the optimum irradiation power. The effect of the extraction time from 7 to 9 min on extraction yields were investigated. There is no significant difference between 7 and 9 min. Hence, 7 min is selected as the optimum extraction time. We investigated the effect of liquid to solid ratio at different ratio of 25:1,30:1,35:1, (ml:g), respectively. TABLE 2 shows that extraction yields change little with increased liquid to solid ratio. Therefore,25:1(ml:g)is chosen as the optimal ratio of liquid to solid. The effect of ethanol concentration on extraction yields were ex-

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amined. The extraction yields increase with increasing ethanol concentration The extraction yields change little when the ethanol concentration increases to 80%(v/v). The reason may be that the solubility of resveratrol in ethanol reaches saturation when the ethanol concentration reaches a certain value. In general, microwave irradiation power, extraction time, ethanol concentration and ratio of liquid to solid, are 1.5 kw, 7 min, 80%(v/v), and 25:1(ml:g),respectively.

Orthogonal test analyses show that the factors affecting the extraction yields are as follows: microwave irradiation power>ethanol concentration>extraction time>ratio of ratio of liquid to solid with the most significant factor being microwave irradiation power. Variance analysis also indicates that the effect of extraction microwave irradiation power is prominent.

To study the stability of the optimum conditions, the optimum experiments were repeated three times. The results indicate that there is good repeatability.

Mass Spectrometry analysis

Extract mass spectrogram was shown in figure 4. $[M-H]^-$ ion peak was at 227.8,which was consistent with resveratrol molecular weight of 228.2. The result showed that the extraction of the samples was resveratrol.

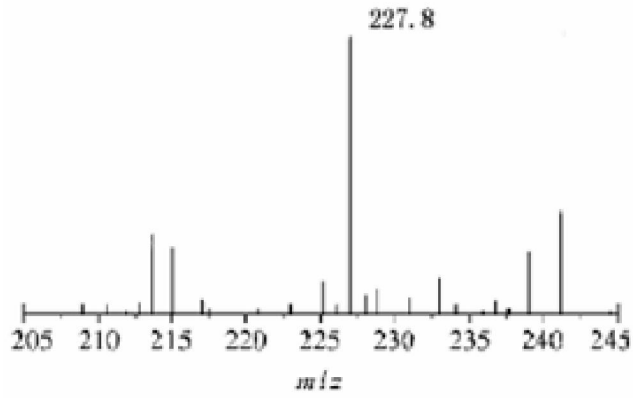

Figure 4 : Extract mass spectrogram

CONCLUSION

In the present study, microwave-assisted extraction technology was used to extraction resveratrol from Polygonum Cuspidatum Sieb et Zucc and the extraction conditions were optimized. Optimized extraction conditions is the microwave irradiation power of 1.5kw, the liquid - solid ratio of 25:1 (ml:g), ethanol concentration of 80%(v/v), extraction time 7 min. This method is environmentally friendly. Microwave-assisted extraction resveratrol from Polygonum Cuspidatum Sieb et Zucc is not only theoretical significance, but the prospects for industrial applications.

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