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Study on the Ultrasonic-Microwave Assisted Extraction Technology of Isoflavonoids and Starch from *Radix puerariae*

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ABSTRACT

After separating starch from pueraria, the residue was used for extraction of pueraria isoflavones and realized the raw material comprehensive utilization as well as the resolver and energy saving. *Radix puerariae* was used as raw material to optimize isoflavones extraction with the aid of microwave and ultrasonic technology. Effects of temperature, solvent, solvent-material ratio, extraction time, microwave power, ultrasonic frequency on extraction rate of isoflavones were evaluated through orthogonal experiments. Results showed that the optimal extraction condition: The starch was removed before the extract, the temperature was 70, the solvent was 70% (v/v) ethanol, the solvent-material ratio was 15:1, the extraction time was 1.0 h, the microwave power was 100 W and the ultrasonic frequency was 50 kHz. The pueraria isoflavones extraction ratio was 92.83%, the purity reached 21.65% and the extraction rate of starch was 42%.

Key words: *Radix puerariae*, ultrasonic extraction, microwave extraction, isoflavones, comprehensive utilization

INTRODUCTION

Radix puerariae is approved edible and medicinal plant by National Ministry of Health which belongs to the drying root of *Pueraria lobata* (Willd) Ohwi or *P. thomsonii* Benth and contains abundant isoflavones and starch materials. *Pueraria* isoflavones proves it has the prevention and amendment of cardiovascular disease, anti-tumor, antioxidant, protecting liver, regulating endocrine system and some other pharmacological effects. The content of *Pueraria* isoflavones reaches as high as 7.6% in *Radix puerariae* which include over thirty kinds of puerarin, daidzin and daidzein. Of them, Puerarin is the major and active ingredient, accounting for about percent 50 (Reppert *et al.*, 2008; Xu *et al.*, 2008; Xu and He, 2007; Ratola *et al.*, 2009).

At present, comprehensive utilization level of pueraria resources is low in our country, starch from *Radix puerariae* as waste often was abandoned in the pharmaceutical industry; While, producers of starch from *Radix puerariae* often ignore the recycling of isoflavones (Zhang and Liu, 2008). Manufacturers of starch from *Radix puerariae* and companies of isoflavonoids from *Radix puerariae* produce large amounts of organic waste water at the same time which seriously

polluted the environment. Rational utilization of *pueraria* resources is imminent. In recent years, ultrasonic and microwave assisted extraction technology was widely applied in the extraction of efficient components plant (Navarro *et al.*, 2009; Chen *et al.*, 2010; Hudaib *et al.*, 2003). After separating starch from *pueraria*, ultrasonic microwave assisted extraction technology of isoflavonoids from *Radix pueraria* was studied, aims at fully comprehensive utilization of puerarin resources rationally, improve the puerarin economic value, to provides the reference for the industrialized production of pueraria powder and isoflavones from *Radix puerariae*.

MATERIALS AND METHODS

Materials: *Pueraria*, puerarin sample, 95% ethanol (AR), methanol (AR), homemade double distilled water (laboratory).

Apparatus: Winnowing Chinese medicine grinder (WKX-160), stainless steel electrothermal blowing display (101-1), one over ten thousand electronic balance (Sartorius), constant temperature water-bath water (HH-S), vacuum pump (SHB-III), rotary evaporation instrument (RE-52AA), high-speed centrifuge (Avanti J25), spectrophotometer (CARY 100).

Extraction technology of isoflavonoids and starch from *Radix puerariae*: *Pueraria* powder was extracted by water first and then ethanol as solvent, using ethanol reflux method in the extraction of isoflavones from *Radix puerariae*.

Resh *pueraria* was cleaned, cut into parts, dried, low temperature drying after being shattered, getting 20 mesh *pueraria* powder. At 40, solid-liquid ratio 1:6, water extraction for 3 h, 100 mesh sieve filtration material. Getting starches from *pueraria* after the separation, centrifugal separation after cooling (4500 r/min, 10 min), supernatant and starch were separated. Starch was dried at 60. The filter residue left was dried at 60, as raw extraction materials for the subsequent isoflavones from *Radix puerariae*. Hundred gram raw material: Getting 60 g starch (moisture content 8%, starch content 8%), 26 g residue (moisture content 5.5%). Residue was dissolved by 95% ethanol and then put into 250 mL ultrasonic-microwave extraction apparatus, set the extraction time and microwave power, the ultrasonic power set at 50 w, extraction was finished, place the extraction liquid cooling and then centrifugal separation (1000 r/min, 30 min), the supernatant fluid was reduced pressure concentration for isoflavones from *Radix puerariae*.

Raw material particle size, extraction time, ethanol to material ratio, solvent concentration, extraction temperature and extraction factors on the influence of extraction rate of isoflavones from *Radix puerariae* was investigated on the basis of the extraction process and then set orthogonal test parameters.

Basic indicators of isoflavones from *Radix puerariae* was determined. Basic indicators of isoflavones from *Radix puerariae* include total isoflavones content, extraction yield of isoflavones and the purity of isoflavones. To optimize the extraction technology parameters, the isoflavone extraction yield as the main evaluation index. Accurately weigh puerarin prototype 50 mg which was dried to constant weight. With 95% ethanol to dissolve until the capacity for 10 mL, get 5 mg mL⁻¹ standard solution of puerarin.

Determination of detection wavelength: Respectively scan in all band 200-900 nm range by UV-vis spectrometer in, the maximum absorption wavelength was detected wavelength.

UV standard curve of puerarin was drawn. Accurately measured puerarin standard solution 0.2, 0.4, 0.6, 0.8 and 1.0 mL, diluted with distilled water to 10 mL, respectively, shaken, let stand waiting for measurement. A 1.0 mL 95% ethanol diluted with distilled water to 10 mL as blank test. Measured at 250 nm absorbance values, parallel determination of 3 times, take the average. With absorbance value as the ordinate, the concentration of puerarin as the abscissa, drawing standard curve.

Preparation and determination of the sample solution: Accurately measured 3.00 g dry puerarin, after the extraction of starch from *Radix puerariae*, placed in the backflow flask. After ultrasonic-microwave assisted extraction, get the

concrete by stress concentration and then dissolved with 95% ethanol, rated capacity, get a certain concentration of sample solution, set aside.

Accurately measured a certain amount of sample solution, according to the proportion, diluted into appropriate concentration solution to be detected. A 1.0 mL 95% ethanol diluted with distilled water to 10 mL as blank test. Measured at 250 nm absorbance values, parallel determination of 3 times, take the average.

$$W_f = (0.111X + 0.0097) \times 10^{-6} \times N \times V \quad (1)$$

where, W_f is total isoflavones content in the sample solution (g), X is Absorbance values of the solution to be detected at 250 nm, N -diluted multiples, V is the volume of the sample solution (mL).

The average absorbance value is 0.4493, calculate the content of isoflavones in 3.00 g *Radix Puerariae* is 0.19749, according to the Eq. 1, the puerarin content of isoflavones from *Radix puerariae* about 6.58%.

Extraction yield of isoflavones:

$$T_f = W_f / (W_0 \times 6.58\%) \times 100\% \quad (2)$$

where, T_f is extraction yield of isoflavones (%), W_f is total isoflavones content in the sample solution (g), W_0 is *Radix Puerariae* dry weight (g).

Purity of isoflavone:

$$P_f = W_f / W_1 \times 100\% \quad (3)$$

where, P_f is the purity of isoflavone, W_f is total isoflavones content in the sample solution (g), W_1 is the quality of the extract (g).

Single factor experiment of extraction process parameters were carried out. Through single factor experimental study on the factors on the influence of extraction rate of isoflavones from *Radix puerariae* to determine the significant influence factors and the best extraction process parameters. The granularity of materials (20 mesh, 40 mesh, 60 mesh, 80 mesh and 100 mesh), extraction time (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h), solvent-material ratio (10:1, 15:1, 20:1, 25:1 and 30:1), solvent concentration (0, 50, 60, 70, 80 and 90%), extraction temperature (50, 60, 70 and 80), microwave power (60, 80, 100, 120 and 140 w).

Determination of optimal extraction process parameters and verification experiments: The extraction of isoflavones from *Radix puerariae* is a complex process of multiple factors interaction. To intuitive analysis of the complicated process, this study will be on the basis of single factor experiment, extraction time, solvent-material ratio and ethanol concentration, extraction temperature on the influence of

isoflavones extraction yield were focused on, extraction experiments according to orthogonal experiment was designed.

RESULTS AND DISCUSSION

UV standard curve of puerarin drawing: With puerarin concentration ($\mu\text{g mL}^{-1}$) as the abscissa, absorbance value as the ordinate mapping, the equation of UV standard curve of puerarin was got. The results is shown in Fig. 1. Figure 1 shows that the equation of UV standard curve of puerarin: $Y = 0.111X + 0.0097$, $R^2 = 0.9998$ ($N = 5$), the concentration of puerarin in the range $2\text{--}10 \mu\text{g mL}^{-1}$, linear is good, the scope of its accurate detection limits in $1.7\text{--}7.1 \mu\text{g mL}^{-1}$.

Results of single factor experiments

Effect of particle size on the extraction rate of isoflavones: The results of isoflavones from *Radix puerariae* extraction yield of different mesh pueraria powder is shown in Fig. 2.

Figure 2 shows that the effect of particle size on the extraction rate of isoflavones is little. In order to save energy and the cost of production, comprehensive consideration, the influence factors in the orthogonal experimental design is no longer considered. A 20 mesh pueraria powder as the research object in the subsequent experiments.

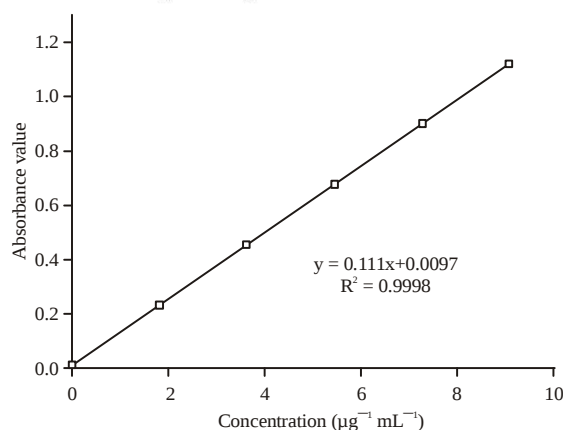


Fig. 1: UV standard curve of puerarin

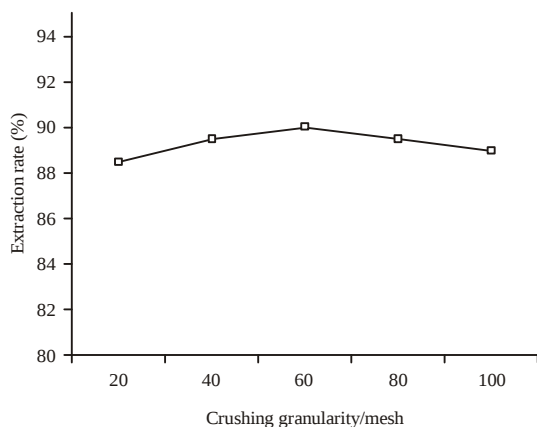


Fig. 2: Effect of particle size on the extraction rate of isoflavones

Effect of ethanol to material on the extraction rate of isoflavones: Twenty mesh pueraria powder was added solvent respectively, solvent-material ratio (10:1, 15:1, 20:1, 25:1, 30:1), other conditions according to the aforementioned method, the extraction yield of the isoflavones was calculated. The results is shown in Fig. 3.

Figure 3 shows that with the increase of solvent-material ratio, the extraction yield of isoflavones increases, increases gently after 25:1. Considering the extraction yield of isoflavones, production costs and the comprehensive utilization of raw materials, solvent-material ratio was selected under 25:1.

Effect of concentration of ethanol on the extraction rate of isoflavones: Twenty mesh pueraria powder was added different concentrations ethanol respectively, ethanol concentrations (0, 50, 60, 70, 80 and 90%), other conditions according to the aforementioned method, the extraction yield of the isoflavones was calculated. The results is shown in Fig. 4.

Figure 4 shows that pure water extraction rate is small, this is because the isoflavones does not dissolve in water. Before the ethanol concentration arrives 70%, with the increase of ethanol concentration, isoflavone extraction rate

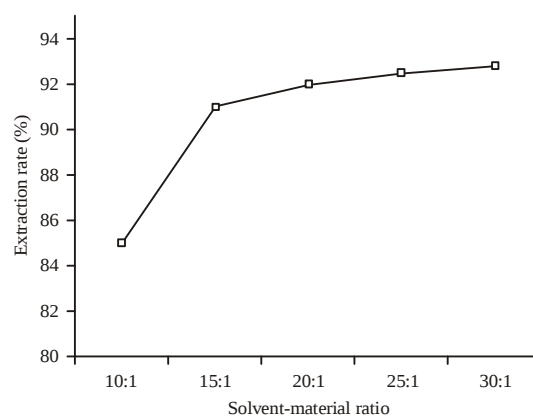


Fig. 3: Effect of ethanol to material on the extraction rate of isoflavones

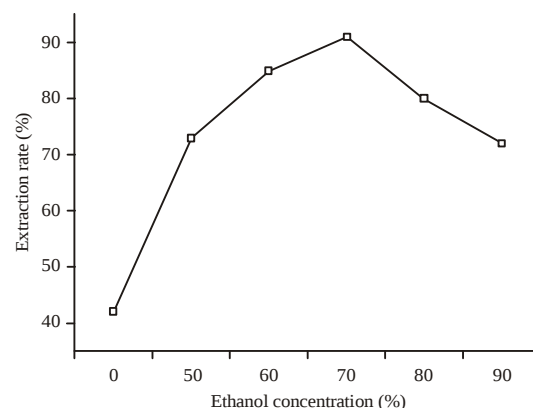


Fig. 4: Effect of concentration of ethanol on the extraction rate of isoflavones

increases. More than 70%, the extraction rate of isoflavones from *Radix puerariae* fell slightly, the reason is that the solubility of isoflavones in different concentrations of ethanol is different. Therefore, choose 70% ethanol concentration is better.

Effect of extraction time on the extraction rate of isoflavones: Twenty mesh pueraria powder was reflux extracted respectively for 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h. Other conditions according to the aforementioned method, the extraction yield of the isoflavones was calculated. The results is shown in Fig. 5.

Figure 5 shows that with the increasing of extraction time, the extraction rate of isoflavones increases. After extracting time more than 2 h, extraction rate increases gently. Considering the extraction time is too long, can make the production cycle extended, causing the cost of operation increases. Therefore, extracting time not more than 2 h in the orthogonal experiments.

Effect of extraction temperature on the extraction rate of isoflavones: Add the 20 mesh pueraria powder in ethanol solution, because the boiling point of ethanol is bottom, in order to avoid the solvent boiling, in this experiment the temperature is 50, 60, 70, 80, other conditions according to the aforementioned method, the extraction yield of the isoflavones was calculated. The results is shown in Fig. 6.

Figure 6 shows that with the increasing of extraction temperature, the extraction rate of isoflavones increases. But when higher than 80, the extraction rate slightly reduces, as the temperature increasing the solubility of isoflavones increases and extraction yield of the isoflavones increases but when the temperature is too high, flavonoids mother nucleus structure easy to be destroyed, the extraction yield declines. Considering the solvent properties, isoflavones extraction yield and production energy consumption, extraction temperature does not exceed 80.

Effect of microwave power on the extraction rate of isoflavones: Microwave extraction of 20 mesh pueraria powder, microwave power respectively selecting 60, 80, 100, 120 and 140 w, other conditions according to the aforementioned method, the extraction yield of the isoflavones was calculated. The results is shown in Fig. 7.

Figure 7 shows that when the microwave power is less than 100 w, the extraction rate of isoflavones increases gradually, when the microwave power is greater than 100 w, the extraction rate of isoflavones is on the decline. Therefore, the suitable microwave power is 100 w.

Determination of optimal extraction process parameters and verification experiments: Using 20 mesh pueraria powder, microwave power 100 w, ultrasonic power 50 w, other conditions according to the aforementioned method, according to the Table 1, design orthogonal experiment, the extraction yield of the isoflavones was calculated.

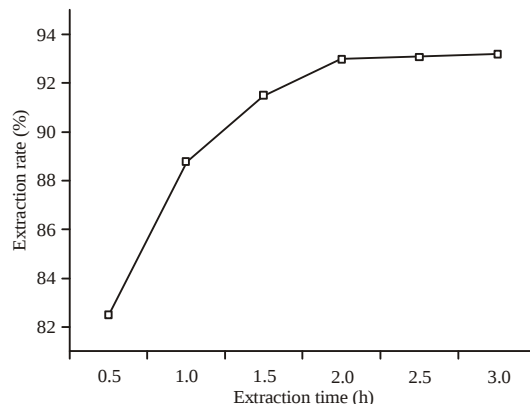


Fig. 5: Effect of extraction time on the extraction rate of isoflavones

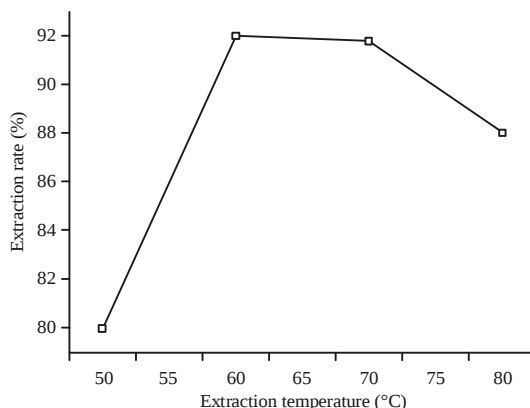


Fig. 6: Effect of extraction temperature on the extraction rate of isoflavones

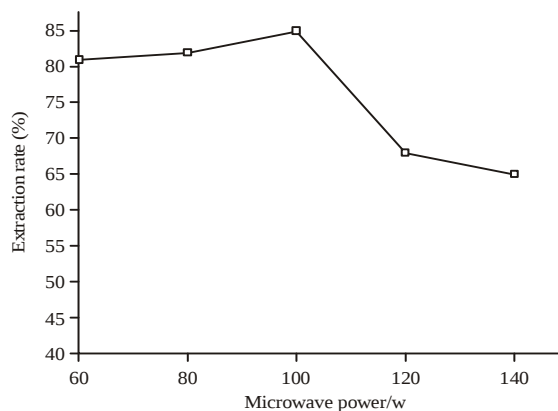


Fig. 7: Effect of microwave power on the extraction rate of isoflavones

Table 2 shows that extraction temperature has the greatest influence on the extraction rate, ethanol concentration the second, solvent-material ratio is minimal. Extract optimum technological conditions was determined for $A_2B_3C_2D_3$, that is the temperature was 70, the solvent was 70% (v/v) ethanol, the

Table 1: Factors and levels of orthogonal table

Level/factors	Solvent-material ratio (g mL ⁻¹)	Ethanol concentration (%)	Extraction time (h)	Extraction temperature (°C)
1	10:1	50	0.5	50
2	15:1	60	1.0	60
3	20:1	70	1.5	70
4	25:1	80	2.0	80

Table 2: Arrangements and results of orthogonal design

No.	A	B	C	D	E	Extraction rate (%)
1	1	1	1	1	1	40.68
2	1	2	2	2	2	84.51
3	1	3	3	3	3	86.78
4	1	4	4	4	4	45.32
5	2	1	2	3	4	91.65
6	2	2	1	4	3	64.34
7	2	3	4	1	2	68.11
8	2	4	3	2	1	61.06
9	3	1	3	4	2	48.61
10	3	2	4	3	1	70.64
11	3	3	1	2	4	89.57
12	3	4	2	1	3	59.17
13	4	1	4	2	3	64.23
14	4	2	3	1	4	54.96
15	4	3	2	4	1	65.96
16	4	4	1	3	2	83.13
k1	64.323	61.293	69.430	55.730	59.585	
k2	71.290	68.612	75.323	74.843	71.090	
k3	66.998	77.605	62.852	83.050	68.630	
k4	67.570	62.170	62.075	56.057	70.375	
R	6.967	16.312	13.248	27.320	11.505	

Table 3: Results of the best extracting technology of isoflavones

No. of test	Extraction rate (%)
1	92.87
2	92.78
3	92.85
4	92.83

solvent-material ratio was 15:1, the extraction time was 1.0 h. Because the combination is not in orthogonal table, this group of extraction conditions was verified. The test results as shown in Table 3.

The average extraction rate of the optimum extraction process was 92.83% which was higher than the highest of 91.65% from orthogonal table, so, the condition was the best process conditions.

Calculation of extraction rate and purity of isoflavones: By the Eq. 2 and 3, the extraction rate and purity of the isoflavones were calculated respectively were 92.83 and 21.65%.

CONCLUSION

Determine the 250 nm was UV wavelength, the equation of UV standard curve of puerarin was drawn, the concentration of puerarin in the range 2-10 µg mL⁻¹, linear is good, the scope of its accurate detection limits in 1.7-7.1 µg mL⁻¹.

Ethanol reflux method was used, the optimum extraction process parameters was determined. The extraction rate of isoflavones from *Radix puerariae* was 92.83%, the purity was 21.65% and the extraction rate of starch was 42%.

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