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Optimization of Ultrasonic-Assisted Extraction of Paeoniflorin from *Radix Paeoniae Alba*

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Ultrasonic-assisted extraction was used to extract paeoniflorin from *Radix Paeoniae Alba*. Single factor experiment and orthogonal array design were used to study the influences of extracting conditions on the yield of paeoniflorin. The content of paeoniflorin from *Radix Paeoniae Alba* was determined by UV spectrophotometry. The optimum extracting parameters were established with the liquid to solid ratio of 15 mL/ g, ultrasonic temperature of 50 °C, ultrasonic time of 10 min, ethanol concentration of 40 %. The optimum technology for ultrasonic-assisted extraction of paeoniflorin from *Radix Paeoniae Alba* is high efficient, extraction yield 8.76 %.

Keywords: Paeoniflorin; *Radix Paeoniae Alba*, Ultrasonic-assisted extraction, Orthogonal array design.

INTRODUCTION

Radix Paeoniae Alba is the dried root of *Paeonia lactiflora* Pall., family Ranunculaceae. It is one of the most widely used Chinese herbal drugs. It can nourish and stabilize the blood, alleviate pain, pacify the liver, edginess, reduce and smooth irritability and mood swings[1]. Paeoniflorin is the major bioactive constituent existing in *Radix Paeoniae Alba*. It has broad pharmacological effects, such as antidiuretic, analgesic, anticonvulsant, antiinflammatory, vasodilatic, *etc*.[1,2].

Paeoniflorin has been extracted from *Radix Paeoniae Alba* by some traditional extraction methods in the past. These include heating reflux extraction[3], homogenate extraction[4,5] and supercritical fluid extraction[6], with water, ethanol and some mixtures as solvents. However, the main disadvantage of the traditional extraction lies in the complicated working procedure which increases the cost; repeated distillations prolong the heating time and accelerate oxidation of the extract. The industrial applicability of supercritical fluid extraction is hindered by the use for complex, expensive apparatus and its low yield of paeoniflorin[6]. Ultrasonic-assisted extraction was an efficient and environment-friendly process or "green" technique. One of the main advantages of ultrasonic extraction over other traditional extraction techniques was its low cost of implementation due to the simplicity of equipment used during sonication processes[7,8]. The use of ultrasound in aqueous extraction of pharmaceutically active ingredients from medicinal herbs is of major interest from a pharmaceutical engineering point of view, since ultrasonic-assisted aqueous extraction technology is an "add on" step[9] which requires minimum alteration of the existing extraction processes[10,11].

In this study, the ultrasonic extraction technology was used to extract paeoniflorin from *Radix Paeoniae Alba*. Single factor experiment and orthogonal array design were used to study the influences of extraction conditions on the yield of paeoniflorin.

EXPERIMENTAL

Ultrasonic cleaning instrument (model: LSA-E24/1200; Front Ultrasonic Technology Co. Ltd., Hangzhou, China); UV spectrophotometer (model: 2450; Shimadzu, Japan); Electronic analytical balance (model: AB204-N; Ohaus, America).

Paeoniflorin was supplied by National Institute for the Control of Phanmaceutical and Biological Products (Beijing, China). *Radix Paeoniae Alba* was purchased from Hangzhou Chinese Medicine Factory (Hangzhou, China). Ethanol (analytical grade) was supplied by Hangzhou Chemical Reagent Co., Ltd. (Hangzhou, China).

Ultrasonic extraction: *Radix Paeoniae Alba* was fried constantly at 60 °C in a vacuum oven and then pulverized (about 40 mesh) with a disintegrator. *Radix Paeoniae Alba* powder (0.5000 g) mixed with extractant (ethanol) was added to a 10 mL centrifugal tube. Setting ultrasonic temperature and time, when the water temperature reaches the set value, put the centrifugal tube into the instrument to begin ultrasonic extraction, with the liquid level in the tube kept lower than of the cleaner tank. Ultrasound energy was supplied at an acoustic frequency of 24 kHz. After the extraction was completed, the extract was filtered .

Preparation of standard stock solution and study of calibration curves: The standard stock solution was prepared by dissolving paeoniflorin in 80 % ethanol to make final concentration of 200 μg mL^{-1}. Different aliquots were taken from stock solution and diluted with 80 % ethanol separately to prepare series of concentrations from 0 to 20 μg mL^{-1}. The λ_{max} was found by UV spectrum of paeoniflorin in ethanol, in the range of 200-400 nm and it was found to be 230 nm. Absorbance was measured at 230 nm against 80 % ethanol as blank. The calibration curve was prepared by plotting absorbance *versus* concentration of paeoniflorin. The calibration curve was shown in Fig. 1.

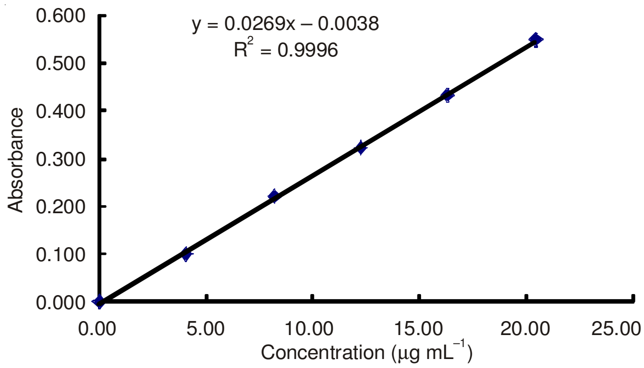

Fig. 1. Standard graph of paeoniflorin by UV spectrophotometric method

Preparation of sample solution: The product of ultrasonic extraction was put into a 50 mL clean dry volumetric flask and dissolved with 80 % ethanol. The obtained solution was used to determine the concentration of paeoniflorin by UV spectrophotometric method. Then the amount of paeoniflorin was calculated.

The yield is defined in eqn. 1. It was measured for each run and averaged for the three values.

$$\text{Yield (\%)} = \frac{\text{g of paeoniflorin extracted}}{\text{g of } \textit{Radix Paeoniae Alba} \text{ powder}} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Influence of ethanol concentration: Ethanol was used as extractant. The influence of ethanol concentration on the yield of paeoniflorin was given in Fig. 2 under other identical extaction conditions. The ethanol concentration was varied from 30 to 60 % while keeping liquid to solid ratio at 10 mL/g,

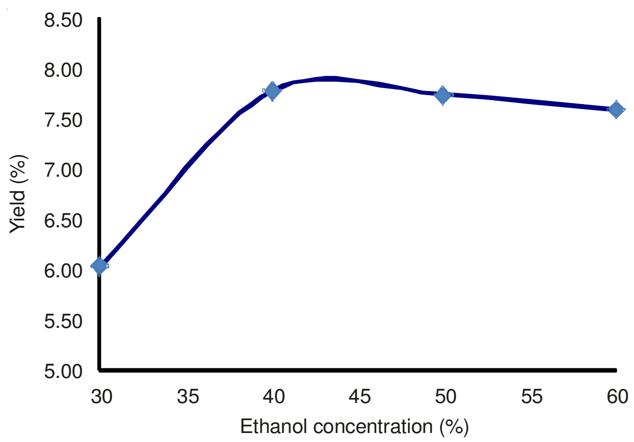

Fig. 2. Effects of ethanol concentration on yield of paeoniflorin

ultrasonic temperature at 50 °C, ultrasonic time at 20 min. The yield of paeoniflorin increases with ethanol concentration, reaching a maximum at 40 % and then declined slightly.

Influence of liquid to solid ratio: The ratio of ethanol to *Radix Paeoniae Alba* powder (mL/g) was varied from 5 to 20 keeping ethanol concentration at 40 %, ultrasonic temperature at 50 °C, ultrasonic time at 20 min. The results are represented in Fig. 3. The yield of paeoniflorin reaches a maximum at 15.

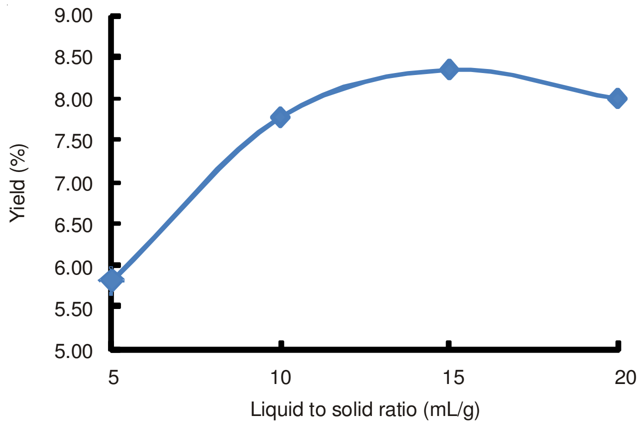

Fig. 3. Effects of liquid to solid ratio on yield of paeoniflorin

Influence of ultrasonic time: The influence of ultrasonic time on yield of paeoniflorin was given in Fig. 4 under other identical extracting conditions. A gradual rise in yield was seen with increase in duration of ultrasonic time. As seen from Fig. 4, in 15 min of ultrasonic time, 8.43 % of yield is obtained and almost no changing thereafter.

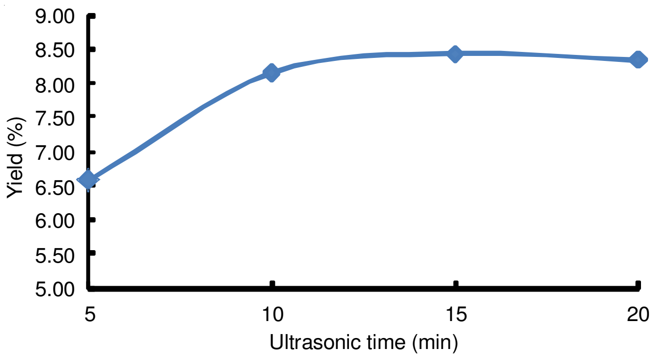

Fig. 4. Effects of ultrasonic time on yield of paeoniflorin

Influence of ultrasonic temperature: The effect of ultrasonic temperature is demonstrated in Fig. 5. Ultrasonic temperature was varied from 45 to 60 °C keeping ethanol concentration at 40 %, liquid to solid ratio at 15, ultrasonic time at 15 min. With the increase in temperature from 45 to 55 °C, the yield of paeoniflorin increases from 6.89 to 8.64 %. With further increase in temperature, the yield of paeoniflorin was decreased.

Orthogonal array design: In order to fully examining the effect of ultrasonic-assisted extraction conditions, an orthogonal array design (OAD) test, L_9 $(3)^4$, was employed as a chemometric method for investigating ultrasonic-assisted extraction of paeoniflorin from *Radix Paeoniae Alba* after

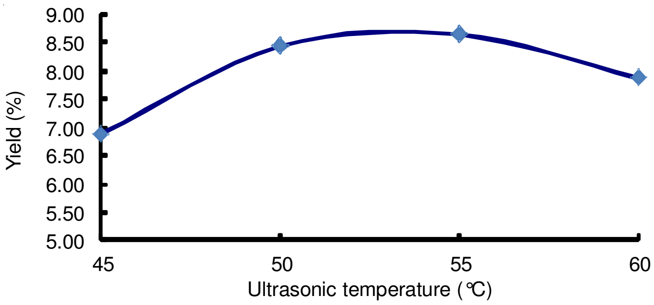

Fig. 5. Effects of ultrasonic temperature on yield of paeoniflorin

single-factor experiments. As listed in Table-1, four processing parameters, namely, liquid to solid ratio (A), ultrasonic temperature (B), ultrasonic time (C) and ethanol comcentration (D), were studied and optimized.

TABLE-1
FACTORS AND LEVELS OF ORTHOGONAL ARRAY DESIGN

Level	A Liquid to solid ratio (mL/g)	B Ultrasonic temp. (°C)	C Ultrasonic time (min)	D Ethanol concentration (h)
1	10	50	10	30
2	15	55	15	40
3	20	60	20	50

The experimental results are listed in Table-2. Ultrasonic temperature was found to be the most important determinant of yield. The results indicated that the optimal conditions for extraction of paeoniflorin from *Radix Paeoniae Alba* by ultrasonic-assisted extraction was $A_2B_1C_1D_2$, namely 15 mL/g of liquid to solid ratio, 50 °C of ultrasonic temperature, 10 min of ultrasonic time and 40 % of ethanol comcentration. Under the optimal conditions, 0.5000 g sample was extracted by ultrasonic-assisted extraction for three times, the average yield of paeoniflorin was 8.76 %.

TABLE-2
RESULTS OF ORTHOGONAL TEST AND ANALYSIS

No.	A	B	C	D	Yield (%)
1	1	1	1	1	7.170
2	1	2	2	2	7.709
3	1	3	3	3	4.512
4	2	1	2	3	7.950
5	2	2	3	1	8.749
6	2	3	1	2	8.192
7	3	1	3	2	8.656
8	3	2	1	3	6.891
9	3	3	2	1	4.902
k1	6.464	7.925	7.418	6.940	
k2	8.297	7.783	6.854	8.186	
k3	6.816	5.869	7.306	6.451	
R	1.833	2.056	0.564	1.735	

Conclusion

The optimum extracting parameters were established with ethanol concentration of 40 %, liquid to solid ratio of 15 mL/g, ultrasonic temperature of 50 °C and ultrasonic time of 10 min. The optimum technology for ultrasonic-assisted extraction of paeoniflorin from *Radix Paeoniae Alba* was high efficient, extraction yield 8.76 %.

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