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Short Communication

Ultrasound-assisted extraction and purification of schisandrin B from *Schisandra chinensis* (Turcz.) Baill seeds: Optimization by response surface methodology

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ABSTRACT

The objective of this study is to develop a process consisting of ultrasonic-assisted extraction, silica-gel column chromatography and crystallization to optimize pilot scale recovery of schisandrin B (SAB) from *Schisandra chinensis* seeds. The effects of five independent variables including liquid–solid ratio, ethanol concentration, ultrasonic power, extraction time, and temperature on the SAB yield were evaluated with fractional factorial design (FFD). The FFD results showed that the ethanol concentration was the only significant factor for the yield of SAB. Then, with the liquid–solid ratio 5 (mL/g) and ultrasonic power 600 W, the other three parameters were further optimized by means of response surface methodology (RSM). The RSM results revealed that the optimal conditions consisted of 95% ethanol, 60 °C and 70 min. The average experimental SAB yield under the optimum conditions was found to be 5.80 mg/g, which was consistent with the predicted value of 5.83 mg/g. Subsequently, a silica gel chromatographic process was used to prepare the SAB-enriched extract with petroleum ether/acetone (95:5, v/v) as eluents. After final crystallization, 1.46 g of SAB with the purity of 99.4% and the overall recovery of 57.1% was obtained from 400 g seeds powder. This method provides an efficient and low-cost way for SAB purification for pharmaceutical industrial applications.

1. Introduction

Schisandra chinensis (Turcz.) Bail., Wu-wei-zi in Chinese, is widely used in Chinese prescriptions as a sedative and tonic to treat various diseases [1]. Currently, *S. chinensis* fruits are widely used as a new dietary ingredient and employed as a natural food preservative and dietary herbal supplement because of its antioxidative, antimicrobial and anti-stress effects [2,3]. *S. chinensis* seeds, a byproduct during the commercial manufacture of juices and wines, are rich in numerous biologically active compounds, such as essential oils, organic acids and lignans [4]. Among the lignans, schisandrin B (SAB) (Fig. 1) is a major component, and the *S. chinensis* seeds contained almost twice the levels of SAB that of fruits [5]. Now pharmacological tests revealed that SAB protects against oxidative brain damage, inhibits P-glycoprotein, and reverses the multiple drug resistance in cancer cells [6,7]. It was observed that recovery of SAB from *S. chinensis* seeds has economical benefits in the both food and pharmaceutical industry.

In recent years, some new methods have been successfully developed for the extraction and isolation of SAB from *S. chinensis* fruits. Solvent fractionation followed by multi-step chromatography was used for purification of the SAB from *Fructus schisandrae* [8]. Preparative high-speed counter-current chromatography was also used for the preparation of SAB from a light petroleum extract of *S. chinensis* fruits with *n*-hexane–methanol–water (35:30:3, v/v) as eluents [9]. One key issue of SAB recovery is how to select a method to increase SAB migration from solid particles at lower temperature, because SAB is not stable in aqueous solution in a heating extraction. Conventional pretreatment in the industrial field may include size reduction, breaking, or grinding with the purpose of debilitating the hard seeds hulls and cell coats in order to enhance the mass transfer coefficient [10]. The reduction in particle size can essentially shorten the processing time, and enhance the overall extraction yield. However, it may cause difficulties in the filtration and raise the cost of processing during the subsequent industry procedures, if the powder of crushed particles is too fine. To overcome this limitation, high-power ultrasound is introduced in this study. Ultrasound has been used to assist the solvent extraction of bioactive compounds from herbs [11–14]. The application of ultrasonic-assisted extraction (UAE) offers many advantages including reduced extraction times, decreased extraction temperature, and increased extraction yields with a desirable size of particles [15]. The enhancement in extraction obtained by

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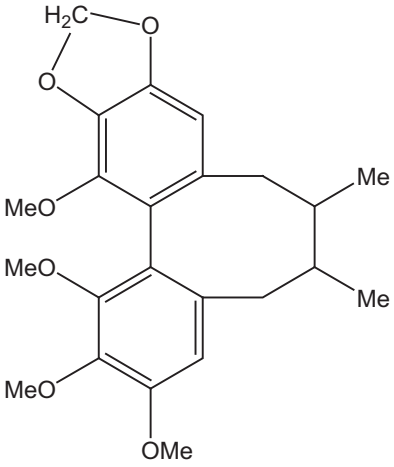

Fig. 1. Structure of schisandrin B.

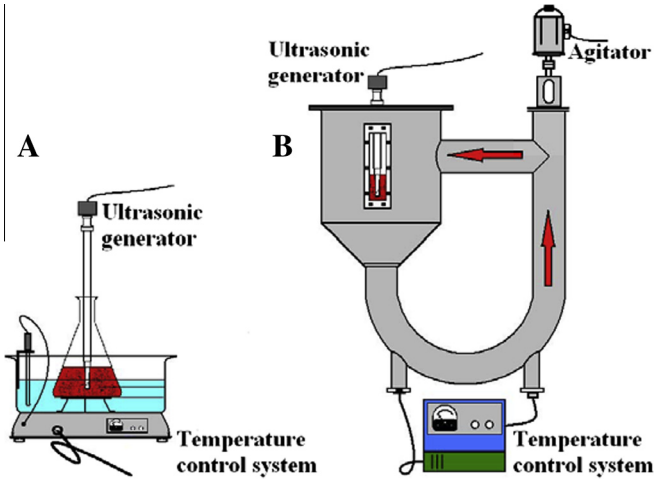

Fig. 2. Ultrasonic extraction equipment, (A) ultrasonic crasher; (B) ultrasonic circulating extraction equipment.

ultrasonic is mainly attributed to the destruction of the cellular wall and enhancement of mass transfer through the cell wall with cavitation effect [16,17].

Experimental design allowed to estimate the effects of several variables simultaneously. The significant factors affecting the UAE on yield of SAB can be deduced, in a screening study, by applying a fractional factorial design (FFD), a powerful tool commonly used in exploratory studies characterized by a large number of potentially influential factors [18–20]. Subsequently, response surface methodology (RSM), which consists of a group of mathematical and statistical techniques, are useful in the modeling and analysis of processes in which a response of interests, such as the yield of activity compounds, is simultaneously influenced by several significant variables [18,21].

The objective of this study is to develop a process to optimize pilot-scale recovery of SAB from *S. chinensis* seeds. First, UAE parameters were determined by FFD, and the operational parameters of the extraction were optimized by RSM. Then, the purification was performed by silica gel chromatography and crystallization.

2. Materials and methods

2.1. Chemicals and herbs

S. chinensis seeds (SAB 6.35 mg/g sample) were obtained from the Wu-wei-zi Cultivation Base of Xiuyan, Liaoning Province, China, in October 2010. *S. chinensis* seeds were ground to powder (0.5–2 mm) using an electrical grinder and stored at 4 °C until use. The SAB standard (No. 110765–200710) was obtained from the National Institute for the Control of Biological and Pharmaceutical Drugs (Beijing, China). All the other chemicals are analytical-reagent grade.

2.2. Apparatus and instruments

The dynamic extraction apparatus used in this work was shown in Fig. 2. An ultrasonic crasher (Fig. 2A) (JY92-II, frequency 40 kHz, maximum to 950 W, Ningbo Scientz Biotechnology Co., Ltd., China) equipped with a cylindrical titanium alloy probe (6.0 mm diameter) was used in the ultrasonic experiments in small scale extraction (5.0–20.0 g powders). An ultrasonic circulating extraction equipment (Fig. 2B) (CTXNW-2B, frequency 40 kHz, maximum to 1000 W, Beijing Hong Xiang Long Co., Ltd.) was employed for pilot scale extraction (400 g powders), and the ultrasound transducer was a horn-type with 15 mm diameter. The working volume of this equipment was 2 L.

2.3. Determination of conditions for UAE in small scale

2.3.1. UAE of SAB from S. chinensis seeds

To optimize the UAE methodology on a small scale extraction, the desired amount of *S. chinensis* seeds powder (6.0–20.0 g) was macerated in a 250 mL glass flask, and the volume of 100 mL aqueous ethanol (30–100%, v/v) was added. The temperature was kept using water bath cauldron. After the extraction was finished, the extract was filtered through 0.45 μm membrane, and the filtrate was held for the determination of yield of SAB (Y) as in Eq. (1).

$$Y\ (\mathrm{mg/g}) = CV/M \tag{1}$$

where C is the measured concentration (mg/mL) of SAB, V is the volume of extraction solvent (mL). M is the mass of *S. chinensis* seeds powder (g).

2.3.2. Experimental design

To achieve the screening of important factors, a 2^5-1 FFD leading to 16 sets of experiments was conducted to verify the most significant factors affecting the SAB yield. The variables were coded according to Eq. (2).

$$x_i = (X_i - X_0)/\Delta X_i \tag{2}$$

Where x_i is the code value of an independent variable, X_i is the real value of an independent variable, X_0 is the real value of an independent variable at the center point, and ΔX_i is the step change value.

The range and the level of the variable both coded values and natural values investigated in present study are all given in Table 1. The SAB yield is considered as the response of Yield (Y).

Based on the information obtained from FFD, a central composite design-CCD also called Box–Wilson design was used in or-

Table 1
Independent variables and their levels employed in FFD.

	Factors	Coded levels		
		−1	0	+1
A	Solvent to solid ratio (v/g)	5	10	15
B	Ethanol concentration (v/v, %)	45	70	95
C	Extraction temperature (°C)	0	30	60
D	Extraction time (min)	30	50	70
E	Extraction power (W)	400	600	800