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Optimization of polysaccharides extraction from seeds of *Pharbitis nil* and its anti-oxidant activity

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ABSTRACT

In the present study, polysaccharides were extracted from *Pharbitis nil* seeds (PNSs) by ultrasonic extraction method for the first time. Response surface methodology with Box–Behnken design was used to optimize the extraction process of PNS polysaccharides (PNSPs). Highest PNSPs yield 6.01 ± 0.15% that agreed closely with the predicted yield 5.99% were obtained under the optimal conditions as follows: ratio of water to raw material 6.5 mL/g, extraction temperature 49.0 °C, ultrasonic power 61.6 W, and extraction time 32.6 min. Preliminary characterization indicated that the sugar, uronic acid and protein contents of the product were 83.6 ± 1.61, 21.8 ± 1.25 and 16.4 ± 0.88% (w/w), respectively. FT-IR analysis revealed the general characteristic absorption peaks of polysaccharides. Besides, PNSPs showed a remarkable antioxidant activity *in vitro* in a dose-dependent manner. These may provide theoretical basis for further system research and rational development and utilization of PNS resources.

1. Introduction

Pharbitis nil Choisy (PN, morning glory), an annual climbing herb that many people use as an ornamental plant, belongs to family *Convolvulaceae* and is widely distributed throughout Southeast Asia (Bensky & Gamble, 1993). The seed of PN has been thought to be one of the most important folk medicine as a purgative drug in China, Korea, and Japan for thousands of years (Bensky & Gamble, 1993). Up to date, it has been demonstrated that the seed is rich in pharbitin (resin glycosides), gibberellins, chlorogenic acid derivatives, anthocyanins, diterpenoids and triterpene saponins that may contribute to the biological functions, such as anti-tumor, anti-fungal, cytotoxic, analgesic and gastroprokinetic activities (Jung et al., 2008; Kim, Jin, Choi, Son, & Lee, 2008; Kim, Chi, & Hong, 2009; Kim, Choi, & Lee, 2009; Ko et al., 2004; Koo et al., 1998; Lee et al., 2008; Saito, Ting, Yokoi, Shigihara, & Honda, 1993; Saito et al., 1994, 1996). However, due to pharbitin with strong purging effect and toxicity, use of PN reduces gradually in clinical application, which causes the waste of PN resources (Huang, 1998). Nowadays, many researches indicated that polysaccharides are slightly toxic and possess a wide range of biological properties, such as immunomodulatory, anti-cancer, hematopoiesis-promoting, and anti-oxidant activities (Li, Wei, You, & Lydy, 2010; Li, Li, et al., 2010; Sarker & Nahar, 2004; Shang et al., 2003; Yang, Jia, Meng, Wu, & Mei, 2006; Yang et al., 2007). To the best of our knowledge, little attention was devoted to PNSPs, especially detailed studies on extraction procedure and its preliminary characteristics.

Recently, ultrasonic extraction (UE) is the new technology that attracts much more attention in the department of extraction in recent years and it is one of the most inexpensive, simple and efficient techniques (Chen et al., 2010; Huang, Xue, Niu, Jia, & Wang, 2009; Yan et al., 2011; Zhang, Yang, Zhao, & Wang, 2009; Zhong & Wang, 2010). The mechanical effect of ultrasound is able to accelerate the extraction of active plant compounds, contained within the body of plants, due to disruption of the cell walls and enhanced mass transfer of cell contents (Hromadková, Ebringerová, & Valachovič, 1999). It offers high reproducibility at shorter times, simplified manipulation, and lowered energy input, as well as solvent consumption (Kim, Chi, et al., 2009; Kim, Choi, et al., 2009; Li, Wei, et al., 2010; Li, Li, et al., 2010; Sun, Liu, Chen, Ye, & Yu, 2011; Vilkhu, Mawson, Simons, & Bates, 2008). Nowadays, response surface methodology (RSM), as an effective tool for optimizing complex processes, is less laborious and time-consuming than

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other approaches (Box & Wilson, 1951). It can reduce the number of experimental trials needed to evaluate multiple variables and their interactions. RSM is easy to arrange and interpret experiment and has already been successfully applied to the optimization of polysaccharide extraction conditions (Chen, Zhang, Jiang, Mu, & Miao, 2012; Chen, Wang, Zhang, & Huang, 2012; Han, Jiang, & Zhang, 2011; Li, Ding, & Ding, 2007; Li et al., 2013; Rodríguez-González, Femenia, Minjares-Fuentes, & González-Laredo, 2012; XuJie & Wei, 2008; Yan et al., 2011; Yongjiang, Zhong, Jianwei, Minger, & Xueqian, 2009).

Therefore, in order to enhance the yield of PNSPs by UE, the extraction variables were optimized by employing RSM. Furthermore, its preliminary characteristics, such as sugar and protein contents, monosaccharide composition and Fourier-transform infrared (FT-IR) spectroscopy, were determined. Besides, the ABTS and DPPH radical scavenging activities were tested *in vitro*.

2. Materials and methods

2.1. Materials

The dry seeds of PN were purchased from a local market (Harbin, China) and identified by Prof. Zhenyue Wang of Heilongjiang University of Chinese Medicine. D-Glucose (Glc), D-glucuronic acid (GlcUA) and sulfuric acid (H_2SO_4) were purchased from Sigma (St. Louis, USA). 1-Phenyl-3-methyl-5-pyrazolone (PMP) was purchased from Kishida (Osaka, Japan). The common chemicals were of analytical reagent grade. All other chemicals were reagent-grade.

2.2. UE of PNSPs

The dried seeds of PN were ground (40 mesh) to obtain a fine powder. For the UE experiments, 100 g of each powder sample was mixed with an appropriate amount of distilled water in a 1000 mL beaker. UE was performed in an ultrasonic device (AS3120A, Tianjin Automatic Science Instrument Co., Ltd, China) working with designed ratio of water to raw material (2, 4, 6, 8 and 10, respectively), extraction temperature (30, 40, 50, 60 and 70 °C, respectively), ultrasonic power (40, 50, 60, 70 and 80 W, respectively), and extraction time (10, 20, 30, 40 and 50 min, respectively). Each extraction processes were performed for 3 times. When the extraction was accomplished, the resulting extract solution was mixed with four times of its volume of 95% ethanol, stirred vigorously and left overnight at 4 °C. And the precipitates were collected by centrifugation (5000 rpm, 10 min), washed with acetone and pure ethanol, and were finally dried at 40 °C to a constant weight. The crude PNSPs obtained was weighted with an analytical balance (AL204, METTLER TOLEDO, Switzerland). PNSP yield was measured using the following equation:

$$\text{PNSP yield} = \frac{\text{weight of crude polysaccharide extract (g)}}{\text{weight of each powder sample (g)}} \times 100\% \quad (1)$$

Table 1
Independent variables and their levels used for Box–Behnken design (BBD).

Independent variables	Symbol	Range and level		
		−1	0	1
Ratio of water to raw material (mL/g)	X_1	4	6	8
Extraction temperature (°C)	X_2	40	50	60
Ultrasonic power (W)	X_3	50	60	70
Extraction time (min)	X_4	20	30	40

2.3. RSM design

Based on the preliminary test results (data not shown), the experimental range of the selected process variables is given in Table 1. Then, a Box–Behnken design (BBD) with four independent variables (X_1, ratio of water to raw material; X_2, extraction temperature; X_3, ultrasonic power; and X_4, extraction time) at three levels was performed, as shown in Table 2. For statistical calculation, the variables were coded by the following equation:

$$X_i = \frac{x_i - x_0}{\Delta x}, \quad i = 1, 2, 3, \quad (2)$$

where X_i is a coded value of the variable; x_i is the actual value; x_0 is the actual value of the independent variable at the center point; and Δx is the step change of variable.

Each variable was prescribed into three levels, coded +1, 0 and −1 for high, intermediate and low value, respectively. The experimental data were fitted to the following second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=0}^{3}\beta_{ii}X_i^2 + \sum_{i=0}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j, \quad (3)$$

where Y is the predicted response; β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively; and X_i and X_j are the coded independent variables.

Analysis of the experimental data and calculation of predicted responses were carried out using Design Expert software (version 8.0, Stat-Ease, Inc., Minneapolis, USA). The analysis of variance (ANOVA) tables were generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significance of each term in the equation is to estimate the goodness of fit in each case. The values of R^2, adjusted-R^2 of models were evaluated to check the model adequacies.

Table 2
BBD and the response values for yields of PNSPs.

Run	Coded variable levels				Yield of PNSPs (%)	
	X_1	X_2	X_3	X_4	Experimental	Predicted
1	−1	−1	0	0	3.74	3.99
2	1	−1	0	0	4.06	4.18
3	−1	1	0	0	3.57	3.66
4	1	1	0	0	4.35	4.31
5	0	0	−1	−1	2.86	2.98
6	0	0	1	−1	3.88	3.75
7	0	0	−1	1	4.54	4.88
8	0	0	1	1	4.86	4.95
9	−1	0	0	−1	3.04	2.99
10	1	0	0	−1	3.36	3.40
11	−1	0	0	1	4.62	4.54
12	1	0	0	1	4.95	4.96
13	0	−1	−1	0	3.88	3.59
14	0	1	−1	0	4.36	4.40
15	0	−1	1	0	5.01	4.92
16	0	1	1	0	3.65	3.90
17	−1	0	−1	0	4.09	3.96
18	1	0	−1	0	4.16	4.08
19	−1	0	1	0	4.16	4.08
20	1	0	1	0	4.83	4.79
21	0	−1	0	−1	3.17	3.27
22	0	1	0	−1	3.15	3.07
23	0	−1	0	1	4.82	4.73
24	0	1	0	1	4.98	4.72
25	0	0	0	0	5.86	5.85
26	0	0	0	0	5.69	5.85
27	0	0	0	0	5.83	5.85
28	0	0	0	0	5.98	5.85
29	0	0	0	0	5.89	5.85