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Optimization of extraction process and antioxidant activity of polysaccharides from leaves of *Paris polyphylla*

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ABSTRACT

Based on a single-factor test, a central composite design was used to optimize the extraction conditions of polysaccharides from leaves of *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz. Three independent variables, including extraction temperature (°C), ratio of water to raw material, and extraction time (h), which significantly affected the yield of polysaccharides, were investigated. The experimental data were fitted to a quadratic polynomial equation using multiple regression analysis and also examined using appropriate statistical methods. The optimum conditions were as follows: extraction temperature, 90.8 °C; ratio of water to raw material, 21.3:1; and extraction time 4.8 h. Under these conditions, the experimental yield was 54.18%, which matched the predicted value well. Furthermore, the purified polysaccharide exerted strong antioxidant effects on DPPH, hydroxyl, and superoxide radicals *in vitro*.

1. Introduction

Paris polyphylla Smith var. *yunnanensis* (Franch.) Hand.-Mazz., which is widely distributed in the southwest of China, has been used as a traditional Chinese medicine for a long time. It is effective on detumescence, pain relief, sore throat, snake bites, and bruises (Wen et al., 2012). Many bioactive ingredients exist in this plant, such as saponin, ecdysone, phytosterol, and flavonoid glycoside (Wu, Gao, & Duan, 2004). They are widely used as antitumor, antimicrobial, hemostatic, and analgesic agents (Qian et al., 2012; Qin et al., 2012; Wang, Xu, & Jiang, 1990). Polysaccharides are polymeric carbohydrate structures, formed of repeating units joined together by glycosidic bonds (Zhu, Heo, & Row, 2010). Polysaccharides have attracted so much attention because of their special physicochemical properties and high biological activities (Forabosco et al., 2006; Yan et al., 2011). However, few studies have been carried out on polysaccharides from leaves of *P. polyphylla* var. *yunnanensis* (PPLPs).

Hot water extraction technology is the common method to extract plant polysaccharides and has been widely used in many researches (Liu, Sun, Liu, & Yu, 2011; Wu, Cui, Tang, & Gu, 2007). In the extraction process, extraction yield is affected by multiple independent variables. Therefore, developing an optimization method that can determine all the factors is necessary. In addition, the possibility of interactions between independent variables should be considered to determine the optimal extraction conditions. Response surface methodology (RSM) is an effective statistical technique in optimizing a process when the independent variables have a combined effect. The main merit of RSM is that the experimental trials to evaluate multiple parameters and their interactions are dramatically reduced. Therefore, RSM needs less materials and time than traditional methods to optimize a process (Giovanni, 1983; Wang & Lu, 2005). Central composition design (CCD) is a typical response surface design. In this design, the treatment combinations are at the experimental spots, axis points, and central points. The design is rotatable (or nearly rotatable) and requires five levels of each factor. CCD is more efficient to conduct experiments compared with traditional methods.

Reactive oxygen species are generated as by-products in the process of cell metabolism. Excessive free radicals can cause many diseases, such as cancer, cardiovascular disease, and atherosclerosis. Conventional medicines for antioxidant and cancer treatment, such as BHA, BHT, and TBHQ, have limited efficacy and significant toxicity (Li & Zhou, 2007). Polysaccharides, a kind of natural biomacromolecule, exist widely in the biological world. Plant polysaccharides have attracted great attention because of their

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Table 1
Independent variables and their levels used in the response surface design.

Independent variables	Level				
	−1.682	−1	0	1	1.682
Extraction temperature (X_1) (°C)	81.59	85	90	95	98.41
Ratio of water to raw material (X_2)	13.18	20	30	40	46.82
Extraction time (X_3) (h)	2.318	3	4	5	5.682

antioxidant, anticancer, and immunobiological activities (Sun et al., 2008; Xu et al., 2009; Ye, Wang, Zhou, Liu, & Zeng, 2008). However, little information is available for PPLPs.

The present study aimed to investigate the key variables (extraction temperature, extraction time, and ratio of water to raw material) and further optimize the process for extraction of PPLPs. In addition, the purified polysaccharide was obtained and its antioxidant activities were evaluated.

2. Materials and methods

2.1. Materials and reagents

The leaves of *P. polyphylla* var. *yunnanensis* were collected from Wenshan, Yunnan Province, China. The dried materials were pulverized to a fine powder in grinder (FW177, Taisite Instrument Co. Ltd.), and then screened though a 60 mesh sieve. The powder was extracted in a Soxhlet apparatus with petroleum ether at 60–90 °C and ethanol for 5 h, respectively. The powder was then dried and reserved in a desiccator at room temperature.

Ethanol, phenol, sulphuric acid, and glucose were purchased from the Chengdu Kelong Chemical Factory (Chengdu, China). 2,2-Diphenyl-1-picryl-hydrazy (DPPH), 1,10-phenanthroline, dihydronicotineamide adenine dinucleotide (NADH), and nitroblue tetrazolium (NBT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

2.2. Extraction of PPLPs

Each pretreated powder (1.0 g) was extracted in water under a designed extraction temperature, ratio of water to raw material, and extraction time. The resulting suspension was separated by centrifugation (5000 rpm, 10 min). The supernatant was collected and added distilled water to 100 ml. The content of polysaccharides was determined using phenol–sulphuric acid method (Cuesta, Suarez, Bessio, Ferreira, & Massaldi, 2003). Glucose was used as standard, and the result was expressed as glucose equivalent.

2.3. Experimental design

A single-factor test was adopted to determine the preliminary range of the extraction variables. A five-level three-factor CCD was used to optimize the best combination of extract variables for the yields of PPLPs. Extraction temperature (X_1), ratio of water to raw material (X_2), and extraction time (X_3) were the independent variables selected to be optimized for the extraction of polysaccharides. The range of three variables and the five levels are shown in Table 1. Extraction yield (Y) was taken as the response for the interaction of three variables in Table 2. 20 experimental runs were carried out at random to minimize the effect of unexpected variability in the observed responses.

Table 2
Central compositional design matrix and response values for polysaccharide yield.

Run	X_1	X_2	X_3	Y (extraction yield, %)
1	0	0	−1.682	51.03
2	0	0	1.682	52.08
3	−1	−1	1	52.79
4	1	−1	−1	52.12
5	−1	−1	−1	48.22
6	0	0	0	53.67
7	0	0	0	53.7
8	−1.682	0	0	45.23
9	1	1	−1	52.69
10	0	0	0	53.5
11	−1	1	−1	45.87
12	1	−1	1	52.6
13	−1	1	1	45.03
14	0	1.682	0	48.94
15	1	1	1	51.56
16	0	–	0	52.83
17	0	0	0	52.99
18	0	−1.682	0	52.31
19	1.682	0	0	51.8
20	0	0	0	53.8

The three variables were coded according to the equation:

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

where X_i was the coded value of independent variable, x_i was the actual value of the independent variable, x_0 was the actual value of independent variable at the central point, and Δx was the step change of the variable. The behavior of the system was explained by the following quadratic equation:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_{ij} \tag{2}$$

where Y was the predicted response, A_0 was a constant, A_i, A_{ii}, and A_{ij} were coefficients estimated by the model, and X_i and X_j were the independent variables. These values represented the intercept, linear, quadratic, and interaction effects of the variables on the response, respectively. The model evaluated the effects of the three variables. All experiments were performed with three replications. To estimate the response of independent variables, the experimental design was analyzed and the predicted data was calculated using Design-Expert software (version 8.0, Stat-Ease, Inc., Minneapolis, USA). Subsequently, three additional experiments were performed to verify the validity of the statistic experimental strategies.

2.4. Preparation and purification of polysaccharides

PPLPs solution from pretreat sample (100.0 g) was extracted using hot water extraction method under the optimized conditions. The suspension was centrifuged at 5000 rpm for 10 min, and the supernatant was then collected and condensed to approximately 100 ml. The protein in the concentrate was removed using Sevag method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). Four volumes of ethanol were added to the concentrate, and the mixtures were held at 4 °C for 12 h. The precipitate was collected and vacuum-dried to obtain PPLPs. PPLPs (1.0 g) was dissolved in deionized water. The solution was membrane-filtered (0.45 μm; Nuclopore) and applied to a DEAE-cellulose DE-52 column (3 cm × 60 cm). After equilibrating with water, the column was eluted with gradient NaCl aqueous solution (0–1.00 mol/ml). The eluates were collected with 10 ml each tube and the total content of polysaccharide was determined using phenol–sulphuric acid method in each tube. Main appropriate fraction (purified polysaccharide) was collected, dialyzed, and lyophilized, and designated