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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

as PPLP. Prior to use, PPLP was dissolved in deionized water and diluted into various concentrations as needed.

2.5. Assay for antioxidant activities

2.5.1. Effect of scavenging DPPH radical

The DPPH free radical scavenging activity was determined by the previously described method (Yang et al., 2009) with minor modifications. In a typical procedure, the reaction solution which consisted of 2 ml sample solution (0.05, 0.1, 0.3, 0.5, 0.7, and 0.9 mg/ml) and 2 ml DPPH ethanol solution (0.1 mmol/l) was incubated for 30 min at 25 °C. The absorbance was measured at 517 nm with a spectrometer (UV-1750; Shimadzu, Kyoto, Japan). DPPH radical scavenging effect was calculated with the following equation:

$$\textit{Scavenging effect}\,(\%) = \left(1 - \frac{A_S}{A_C}\right) \times 100$$

where A_S was the absorbance of a mixture of sample and DPPH solutions, and A_C was the absorbance of the control reaction in which the sample was replaced by ethanol.

2.5.2. Hydroxyl radical assay

The hydroxyl radical assay was determined according to the previously described method (Sun, Wang, Shi, & Ma, 2009) with minor modifications. In a typical procedure, 1.0 ml sample solution (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml) was mixed with phenanthroline (5 mM, 1.0 ml), phosphate buffer (50 mM, PH 7.4), ferrous sulphate (7.5 mM, 0.5 ml), and H_2O_2 (0.1%, 0.5 ml) at 37 °C for 1 h. The absorbance of the mixture was measured at 510 nm with a spectrometer (UV-1750; Shimadzu, Kyoto, Japan). The hydroxyl radical scavenging effect was calculated with the following equation:

$$\textit{Scavenging effect}\,(\%) = \left(1 - \frac{A_S}{A_C}\right) \times 100$$

where A_S was the absorbance of a mixture of sample and reaction solution, and A_C was the absorbance of the control reaction in which the sample was replaced by deionized water.

2.5.3. Superoxide radical assay

The scavenging ability of superoxide radical was measured by the previously described method (Robak & Gryglewski, 1988) with some minor modifications. In a typical procedure, PPLP was dissolved in water, and a series of sample solutions was diluted with different concentrations (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml). An aliquot of each sample solution (1 ml) was mixed with 1 ml of 16 mM Tris–HCl (PH 8.0) containing 557 μM NADH, 1 ml of 16 mM Tris–HCl (PH 8.0) containing 108 μM NBT, and 1 ml of 16 mM Tris–HCl (PH 8.0) containing 45 μM PMS. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance was measured at 560 nm. The scavenging effect was calculated with the following equation:

$$\textit{Scavenging effect}\,(\%) = \left(1 - \frac{A_S}{A_C}\right) \times 100\%$$

where A_s was the average absorbance value of the sample, and A_c was the average absorbance value of the control.

3. Results and discussion

3.1. Single-factor experimental analysis

3.1.1. Effect of extraction temperature on yield of PPLPs

To investigate the influence of temperature on extraction efficiency, extraction process was carried out at 60, 70, 80, 90, and 100 °C, when the extraction time was 3 h and the ratio of water to raw material was 30:1. The extraction yield increased when temperature increased from 60 °C to 90 °C. As shown in Fig. 1(a), the extraction yield increased up to its maximum amount of 49.15% at 90 °C, and then no longer increased when extraction temperature continued to rise. Therefore, temperature range of 85–95 °C was selected as optimal in the CCD experiment.

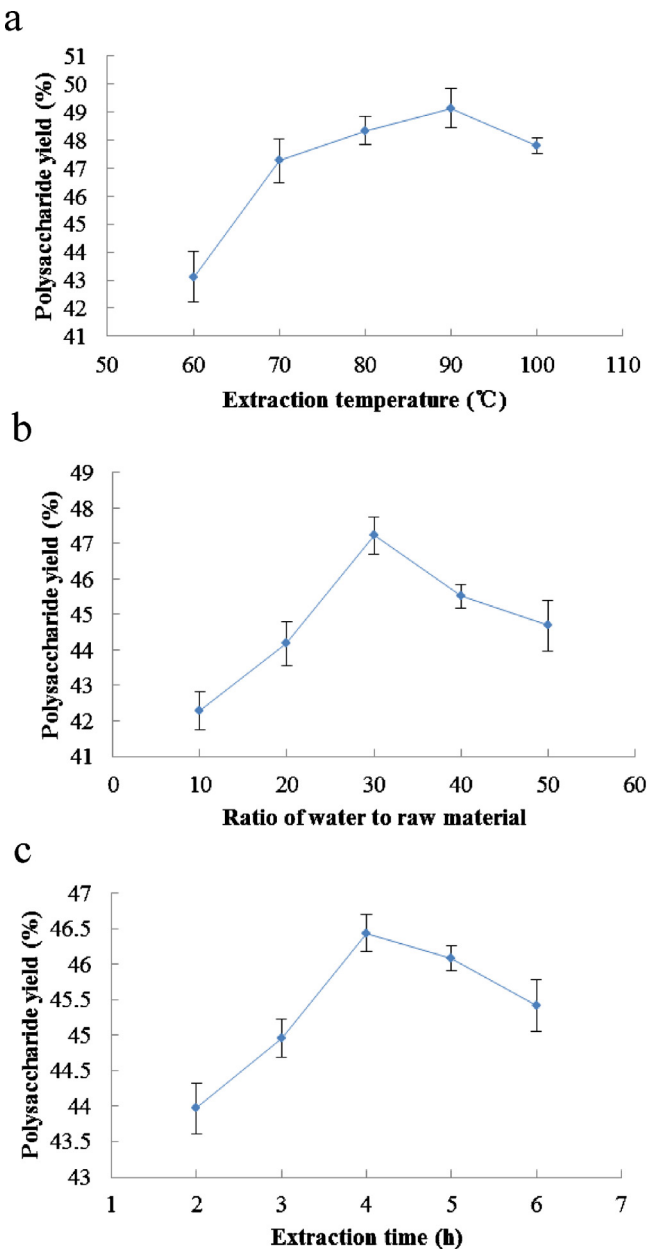

Fig. 1. Effects of extraction temperature (a), ratio of water to raw material (b) and extraction time (c) on the yield of PPLPs.

3.1.2. Effect of extraction time on yield of PPLPs

Extraction time is another factor that influenced the extraction efficiency. Extraction process was carried out at 2, 3, 4, 5, and 6 h, when the ratio of water to raw material was 30:1 at 80 °C. The effect of extraction time on the yield is presented in Fig. 1(b). When extraction time varied from 2 h to 6 h, the extraction efficiency increased and PPLPs yield reached a maximum of 46.44% at 4 h, and then decreased with time. The result indicated that time range of 4–6 h was considered to be optimal in the present experiment.

Table 3
Analysis of variance for the response surface quadratic model.

Source	Sum of squares	Degree of freedom	Mean square	*F*-Value	*p*-value Prob. > *F*
Model	153.26	9	17.03	54.75	<0.0001[a]
X_1	57.86	1	57.86	186.01	<0.0001[a]
X_2	19.33	1	19.33	62.15	<0.0001[a]
X_3	1.72	1	1.72	5.53	0.0406[b]
X_1X_2	11.62	1	11.62	37.35	0.0001[a]
X_1X_3	2.4	1	2.4	7.71	0.0196[b]
X_2X_3	6.16	1	6.16	19.8	0.0012[b]
X_1^2	42.5	1	42.5	136.64	<0.0001[a]
X_2^2	13.6	1	13.6	43.71	<0.0001[a]
X_3^2	5.95	1	5.95	19.13	0.0014[b]
Residual	3.11	10	0.31	–	–
Lack of Fit	2.29	5	0.46	2.77	0.1437[ns]
Pure Error	0.82	5	0.16	–	–
Cor Total	156.37	19	–	–	–
R^2	0.9801	–			–
Adj R^2	0.9622	–	–	–	–
Pred R^2	0.8632	–	–	–	–
Adeq precision	21.095	–	–	–	–
C.V.%	1.09	–	–	–	–

[ns] Not significant.
[a] Significant at $p < 0.001$.
[b] Significant at $P < 0.05$.

3.1.3. Effect of ratio of water to raw material on yield of PPLPs

Ratio of water to raw material, an important extraction parameter, can significantly affect the extraction efficiency. In the present study, ratio of water to material was set at 10:1, 20:1, 30:1, 40:1, and 50:1, when other parameters were as follows: extraction temperature 80 °C and extraction time 3 h. As shown in Fig. 1(c), the extraction efficiency increased up to its maximum amount of 47.22% at 30:1, and then decreased when ratio of water to raw material continued to rise. Thus, ratio of water to raw material range of 20:1 to 40:1 was favorable for extracting PPLPs.

3.2. Optimization of extraction conditions of PPLPs

3.2.1. Predicted model and statistical analysis

The design matrix and corresponding results of RSM experiments to evaluate the three independent variables including extraction temperature (X_1), ratio of water to raw material (X_2), and extraction time (X_3), are presented in Table 2. Based on multiple regression analysis on CCD experimental data, the predicted model was expressed by the following quadratic polynomial equation:

$$Y = 53.41 + 2.06X_1 - 1.19X_2 + 0.35X_3 + 1.21X_1X_2 - 0.55X_1X_3 - 0.88X_2X_3 - 1.72X_1^2 - 0.97X_2^2 - 0.64X_3^2 \quad (3)$$

where Y was the predicted yield of polysaccharides, and X_1, X_2, and X_3 were the coded parameters for extraction temperature, ratio of water to raw material, and extraction time, respectively.

Analysis of variance (ANOVA) was performed to evaluate the predictive model and the variables. The *p*-values were used as a tool to check the significance of each coefficient and indicated the pattern of interactions between variables. The smaller the value of p was, the more significant the corresponding coefficient was. A significant lack of fit demonstrated that the fitted model failed to represent the data in the experimental domain at which points were not included in the regression. The ANOVA for the fitted quadratic polynomial model of extraction efficiency is presented in Table 3. The "Model *F*-Value" of 54.75 implied the model was significant. There was only a 0.01% ($p < 0.0001$) chance that a "Model *F*-Value" this large could occur due to noise. At the same time, the "Lack of Fit *F*-Value" of 2.77 implied the Lack of Fit was not significant relative to the pure error. A "Lack of Fit *F*-Value" this large had 14.37% ($p = 0.1437$) chance to occur because of noise. Coefficient of determination (R^2) indicated that 98.03% of the variations can be explained by the fitted model. The value of adjusted determination coefficient (R^2_{adj}) was 0.9626, which also confirmed that the model was highly significant. Moreover, a relatively low value of coefficient variation (1.09%) indicated high degree of precision and good deal of reliability for the experimental values. "Adeq Precision" measured the signal to noise ratio. A ratio greater than 4 was desirable. The ratio of 21.10 indicated an adequate signal. Thus, the results indicated that this model can be used to navigate the design space.

The regression coefficient values of Eq. (3) are shown in Table 3. All the coefficients were significant ($p < 0.05$), including linear term coefficients (X_1, X_2 and X_3), interaction term coefficients (X_1X_2, X_1X_3 and X_2X_3), and quadratic term coefficients (X_1^2, X_2^2 and X_3^2). The empirical model was converted to three-dimensional (3D) and contour plots to predict the relationships between the independent variables and the response.

3.2.2. Optimization of extraction procedure

The graphical representation of regression Eq. (3) was obtained using Design–Expert to evaluate the effects of independent variables and their interactions on extraction efficiency of PPLPs. In response surface plots and contour plots, the extraction

Table 4
Predicted and experimental values of the responses at optimum and modified conditions.

	Extraction temperature (°C)	Ratio of waterto raw material	Extraction time (h)	Yield of PPLPs (%)
Optimum conditions	90.83	21.3:1	4.8	54.24 (predicted)
Modified conditions	90.8	21.3:1	4.8	54.18 ± 0.072 (actual)

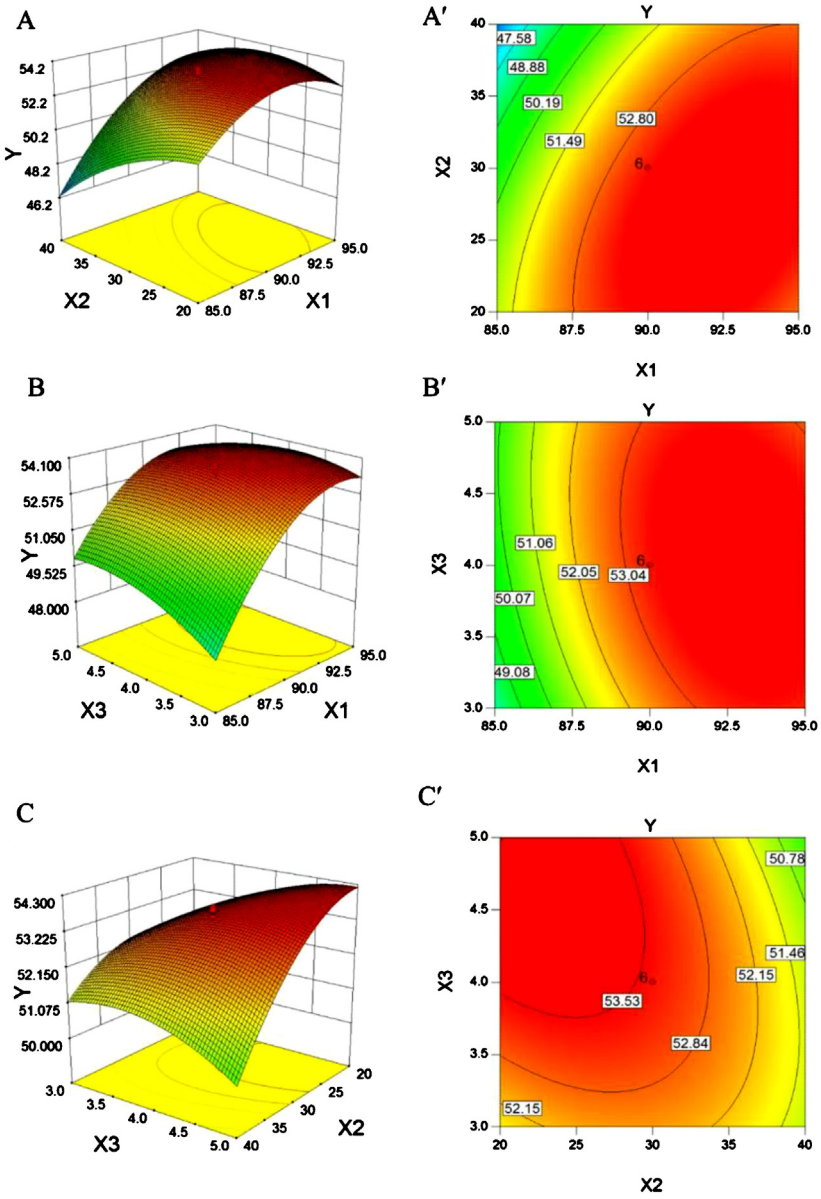

Fig. 2. Response surface plots and contour plots showing the effects of variables (X_1: extraction temperature, X_2: ratio of water to raw material, and X_3: extraction time) on extraction yield of PPLPs.

efficiency of PPLPs was investigated along with two continuous variables, whereas the other one variable was kept at its zero level. These results of the extraction efficiency affected by extraction temperature, ratio of water to raw material, and extraction time are shown in Fig. 2. The extraction yield of polysaccharides affected by extraction temperature and ratio of water to raw material is shown in Fig. 2(a) and (a′) with extraction time fixed at a zero level (4 h). The yield of polysaccharides increased evidently with the extraction temperature from 85 °C to 89.35 °C. However, beyond 89.35 °C, the extraction yield decreased very slowly as the temperature ascended. The response surface and contour plots based on extraction temperature and extraction time are shown in Fig. 2(b) and (b′) with ratio of water to raw material fixed at a zero level (30:1). An increase of polysaccharides yield could be significantly accompanied with the increase of extraction temperature. The extraction yield increased rapidly with the increase of time from 3 h to 4.5 h. However, beyond 4.5 h, the extraction yield increased very slowly with time. The response surface and contour plots based on ratio of water to raw material and extraction time are shown in Fig. 2(c) and (c′) with extraction temperature fixed at a zero level (90 °C). It indicated that the yield of polysaccharides was increased with the ratio of water to raw material from 20:1 to 26:1, and then decreased with the ratio of water to raw material from 26:1 to 40:1.

Based on analysis of single-factor experiments and the plots, the optimal conditions for yield of polysaccharides were as follows: extraction temperature 90.83 °C, ratio of water to raw material 21.3:1, and extraction time 4.8 h. Among the three test independent variables, extraction temperature was the most significant factor to influence the yield of polysaccharides, followed by extraction time and ratio of water to raw material according to the regression coefficient significance of the quadratic polynomial model (Table 3) and gradient of slope in the response surface plots (Fig. 2).

3.3. Verification of the predictive model

To validate the adequacy of the model equation, suitability for predicting optimum values was tested under the selected

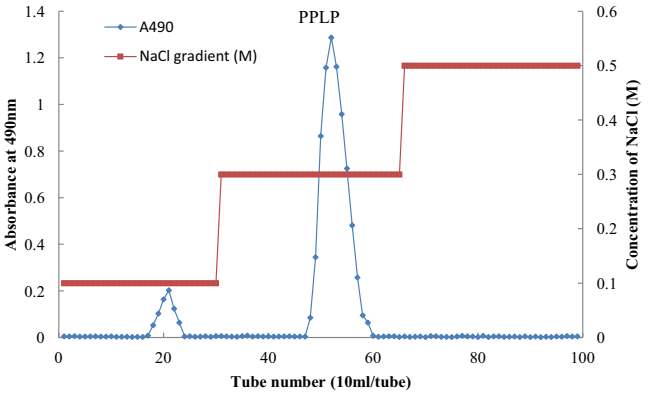

Fig. 3. Elution curve of PPLP by a DEAE-cellulose DE-52 column.

optimal conditions. As shown in Table 4, the optimized extraction conditions were extraction temperature 90.83 °C, ratio of water to raw material 21.3:1, and extraction time 4.8 h, and the predicted yield of PPLPs was 54.24%. To ensure the predicted result not biased towards the practical value, three verification experiments were carried out under the modified optimal conditions: extraction temperature 90.8 °C, ratio of water to raw material 21.3:1, and extraction time 4.8 h. The experimental yield of PPLPs was 54.18% and close to the predicted value. The result indicated no significant difference between experimental and predicted values and confirmed that response model was accurate and adequate for the extraction of PPLPs.

3.4. Isolation and purification of polysaccharides

PPLPs were prepared under the optimal conditions, precipitated by ethanol, deproteinized, and lyophilized. The crude polysaccharide, a brownish powder, presented negative iodine–potassium iodide reaction, which indicated the absence of starch-type polysaccharide. PPLPs were purified by DEAE-cellulose DE52 column chromatography. As shown in Fig. 3, the main peak was eluted with 0.3 mol/l NaCl solution. The main fraction (PPLP) was collected, concentrated, dialyzed, and lyophilized for further study on antioxidant activity. PPLP appeared as a light yellow powder and showed negative response to the ninhydrin test. The fact that no absorption was detected by UV spectra at 260 and 280 nm, implied that PPLP did not contain nucleic and protein.

3.5. Antioxidant activity

3.5.1. Scavenging activity on DPPH radical

DPPH radical is a stable free radical, which has been widely used to evaluate the free radical scavenging activities of natural compounds. Alcohol solution of DPPH has a characteristic absorption maximum at 517 nm. When DPPH ethanol solution is reduced, absorbance is decreased and the solution changes from purple to light yellow. The mechanism of scavenging DPPH radical is caused by the fact that natural compounds can transfer either an electron or a hydrogen atom to DPPH (Naik et al., 2003).

On the basis of this principle, the DPPH radical scavenging activities of PPLP were measured, and the results are shown in Fig. 4(a). The scavenging ability of PPLP was in a dose-dependent manner. The scavenging effect of PPLP increased from 7.62% to 84.73% when the concentration of PPLP increased from 0.05 mg/ml to 0.9 mg/ml. Half inhibition effect (IE_{50}) values for scavenging DPPH radical were 0.25 and 0.06 mg/ml for PPLP and ascorbic acid, respectively. The scavenging activity of PPLP was lower than that of ascorbic acid, and the similar results have been revealed in

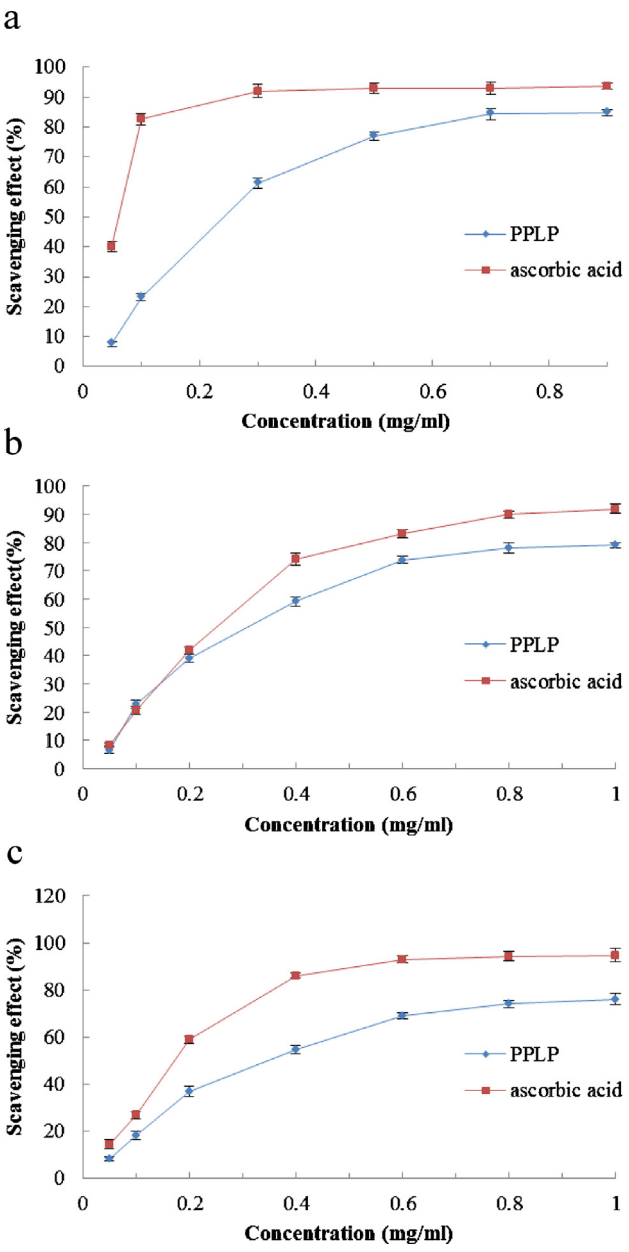

Fig. 4. Scavenging effects of PPLP on DPPH radical (a), hydroxyl radical (b) and superoxide radical (c).

other plant polysaccharides (Ye & Huang, 2012; Yuan, Zhang, Fan, & Yang, 2008; Zha et al., 2009).

3.5.2. Hydroxyl radical scavenging activity

Among the reactive oxygen species, hydroxyl radical is considered to be one of the most potent oxidants which can easily cross cell membranes and readily react with most biomacromolecules, such as carbohydrates, proteins, lipids, and DNA. Hydroxyl radical can cause severe damage to adjacent biomolecules or cell death (Fan, Li, Deng, & Ai, 2012). Therefore, scavenging hydroxyl radical is extremely important for the protection of living systems.

Scavenging effects of PPLP on hydroxyl radical were determined based on the principle of Fenton reaction, and the results are shown in Fig. 4(b). Hydroxyl radical scavenging effects of PPLP increased with concentrations. At concentration of 1.0 mg/ml, scavenging effects on hydroxyl radical were 79.04% and 91.98% for PPLP and ascorbic acid, respectively. IE_{50} values of PPLP and ascorbic acid for scavenging hydroxyl radical were 0.31 and 0.25 mg/ml,

respectively. Results indicated that PPLP had strong capability of scavenging hydroxyl radical, which was close to ascorbic acid. The possible mechanism may involve the supply of either an electron or a hydrogen atom by polysaccharides, which can combine with hydroxyl radical and form a stable compound to terminate the radical chain reaction. Another antioxidant mechanism may be due to supplying either an electron or a hydrogen atom by polysaccharides, which could combine with radical ions such as Fe^{2+} and Cu^{2+}. These mechanisms imply that PPLP may act as electron or hydrogen donor to scavenging hydroxyl radical (Chen et al., 2011; Macdonald, Galley, & Webster, 2003).

3.5.3. Superoxide radical scavenging activity

Superoxide radical is considered as an initial free radical and formed from mitochondrial electron transport system. In addition to directly attacking some biological molecules, superoxide radical can degrade to create other radicals, such as hydroxyl, singlet oxygen, and H_2O_2, which can trigger peroxidation of lipids and induce pathological incidents, such as arthritis and Alzheimer's disease (Liu, Wang, Pang, Yao, & Gao, 2010). Thus, scavenge superoxide radical is necessary.

Superoxide radical was generated by a PMS/NADM system for assay in the reduction of NBT. Scavenging effects of PPLP on superoxide radical are shown in Fig. 4(c). Scavenging activities of PPLP followed a dose-dependent manner at all tested concentrations. The superoxide radical scavenging effects of PPLP and ascorbic acid were 76.09% and 94.78%, respectively, at concentration of 1.0 mg/ml. Moreover, IE_{50} values for scavenging superoxide were 0.35 and 0.17 mg/ml for PPLP and ascorbic acid, respectively. The results indicated that PPLP had a noticeable superoxide radical scavenging activity. However, the scavenging effect of PPLP on superoxide radical was lower than that of ascorbic acid.

4. Conclusions

RSM was confirmed to be a useful tool for the optimization of PPLPs. The optimum variables 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 corresponded with the predicted value well. Additionally, PPLP was obtained by DEAE cellulose-52 anion-exchange chromatography, and it is a light yellow powder. PPLP exhibited strong antioxidant activity *in vitro*. Thus, PPLP should be explored as a novel potential antioxidant, and further studies are essential to evaluate antioxidant activities *in vivo* and elucidate the antioxidant mechanism.

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