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Extraction of polysaccharides from herbal *Scutellaria barbata* D. Don (Ban-Zhi-Lian) and their antioxidant activity

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ABSTRACT

The response surface methodology was employed to study the extraction of polysaccharides from *Scutellaria barbata* D. Don. The quantitative effects of extraction temperature, time, number and ratio of water to raw material on yield of polysaccharides were investigated with Box–Behnken design. The experimental data were fitted to a second-order polynomial equation using multiple regression analysis and also analyzed using the appropriate statistical methods. By solving the regression equation and analyzing 3D plots, the optimum condition was at extraction temperature 70 °C, time 3 h, numbers 3 and ratio of water to raw material 18.5 mL/g. Under these conditions, the experimental polysaccharides yield was 2.43 ± 0.11%, which was in good agreement with the predicted value. The antioxidant activities of the polysaccharides were evaluated in vitro. A potential antioxidant activity of *S. barbata* polysaccharides provides a scientific basis for the use of this herb in traditional medicine as an antioxidant.

1. Introduction

Scutellaria barbata D. Don is a perennial herb which is natively distributed throughout Korea and southern China. This herb is known in traditional Chinese medicine as Ban-Zhi-Lian and traditional Korean medicine as Banjiryun, respectively, and has been used as an anti-inflammatory and antitumor agent (Rugo et al., 2007; Suh et al., 2007; Wong, Lau, Yamasaki, & Teel, 1993). *S. barbata* is known to contain a large number of flavonoids and polysaccharides (Zheng, Wei, & Long, 2010). In the past several years, *S. barbata* flavonoids have been widely studied for their chemical properties and biological activities (Hu et al., 2008; Kim et al., 2005; Lee et al., 2004; Sato et al., 2000; Shi et al., 2011; Wang et al., 2010). Whereas, little attention was devoted to the extraction of the *S. barbata* polysaccharides (SBP), therefore, we reported the optimization of extracting parameters for the production of SBP.

Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Giovanni, 1983). Recently, it has been widely used to develop, to improve, or to optimize a product or process in many cases (Gan, Abdul Manaf, & Latiff, 2010; Gan & Latiff, 2011; Karacabey & Mazza, 2010; Paucar-Menacho, Berhow, Mandarino, de Mejia, & Chang, 2010; Sun, Liu, & Kennedy, 2010; Zhong & Wang, 2009). As a very popular class of designs for fitting a response surface, Box–Behnken design (BBD) provides efficient solutions compared with a three-level full-factorial design, reducing the number of required experiments by confounding higher-order interactions, which becomes more significant as the number of factors increases (Borkowski, 1995; White, Willis, Keshav, & Dutton, 2001).

Free radicals, chemical reactions and several redox reactions of various compounds may cause protein oxidation, DNA damage and lipid peroxidation in living cells (Kil et al., 2009; Sahreen, Khan, & Khan, 2010). In order to reduce damage to the human body and prolong the storage stability of foods, antioxidants are often used for industrial processing. There are two basic categories of antioxidants: synthetic and natural. The use of synthetic antioxidants is restricted because of their carcinogenicity (Guyton et al., 1991; Kimmel, Kimmel, & Frankos, 1986). Thus, there has been increasing interest in finding natural, effective, and safe antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella, Frankel, German, & Kanner, 1993; Nandita & Rajini, 2004). Published data indicate that polysaccharides isolated from plants have certain antioxidant activity on free radicals and can be explored as novel potential antioxidants (Chen, Zhang, Jiang, Mu, & Miao, 2012; Capek, Machová, & Turjan, 2009; Luo et al., 2010).

The purpose of the present study was to optimize the process for production of polysaccharides from *S. barbata*, using response surface methodology (RSM) to study the effects of extraction temperature, extraction time, number of extraction, and ratio of water

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to raw material on the extraction yield of SBP, and the antioxidant activities on superoxide, DPPH radicals and hydroxyl radicals were evaluated.

2. Materials and methods

2.1. Materials

S. barbata were purchased from Bozhou, Anhui Province, China. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Biochemika AG (Buchs, Switzerland) and ascorbic acid (Vc) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Extraction of crude polysaccharides

Dried *S. barbata* (30.0 g) were ground in a high speed disintegrator (Model SF-2000, Chinese Traditional Medicine Machine Works, Shanghai, China) to obtain a fine powder, then were extracted in a Soxhlet apparatus with aether (20–40 °C), and pretreated with 80% aether twice to remove some colored materials, oligosaccharides and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained, as described previously (Yang, Qu, & Cheng, 2004; Zykwinska, Rondeau-Mouro, Garnier, Thibault, & Ralet, 2006). The pretreated dry powder (30.0 g) was extracted with deionized water (water-material (mL/g) ranging from 15:1 to 25:1) at pH 6.5–7.5 (adjusting the suspension pH by 0.1 mol/L NaOH or HCl), while the temperature of the water bath was kept steady for a given temperature (within ±1.0 °C, extraction temperature ranging from 50 to 70 °C). The water-material slurry in a 2.0 L stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 1 to 3 h) during the entire extraction process. The extracted slurry was centrifuged at 4200 rpm/min for 20 min to collect the supernatant, and the insoluble residue was treated again (extraction times ranging from 1 to 3) as mentioned above.

The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (Senco Technology and Science Inc., Shanghai, China) at 55 °C under vacuum. The resulting solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept overnight at 4 °C. Then the solution was centrifuged at 4200 rpm/min for 20 min, washed six times with dehydrated ethanol, and the precipitate was collected as crude extract. The extract was air-dried at 50 °C until its weight was constant, and then was weighted with a balance (BS2202S, SARTORIUS, Germany). The percentage polysaccharides yield (%) is calculated as follows:

$$\text{polysaccharides yield (\%, w/w)} = \frac{\text{dried crude extraction weight}}{\text{powder weight (30 g)}} \times 100$$

2.3. Experimental design and statistical analysis

A four-variable, three-level Box–Behnken design (BBD) (Ravikumar, Pakshirajan, Swaminathan, & Balu, 2005; Wang, Sun, Cao, Tian, & Li, 2008) was employed in this optimization study based on the results of preliminary experiments. Extraction temperature (°C, X_1), extraction time (h, X_2), extraction number (X_3), and ratio of water to raw material (mL/g, X_4) were the independent variables selected to be optimized for the extraction of *S. barbata* polysaccharides. Each variable set at the three levels. Extraction yield (Y) was taken as the response of the design experiments. The coded and uncoded (actual) levels of the independent variables are given in Table 1. Twenty-seven experiments were augmented with three times and carried out at the center points to evaluate the pure error.

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

Independent variables	Symbol	Factor level		
		−1	0	1
Extraction temperature (°C)	X_1	50	60	70
Extraction time (h)	X_2	1	2	3
Extraction number	X_3	1	2	3
Ratio of water to raw material (mL/g)	X_4	15	20	25

A second-order polynomial regression model was used to express the yield as a function of the independent variables as follows:

$$Y = \alpha_0 + \sum_{i=1}^{4}\alpha_i X_i + \sum_{i=1}^{4}\alpha_{ii}X_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{4}\alpha_{ij}X_iX_j \quad (1)$$

where Y represents the response variables, α_0 is a constant, α_i, α_{ii} and α_{ij} are the linear, quadratic and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables.

The fitted polynomial equation is expressed as surface plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Lu, Engelmann, Lila, & Erdman, 2008). The analysis of variance tables was generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The regression coefficients were then used to make statistical calculation to generate dimensional maps from the regression models. Design-Expert 8.0.5b (Trial Version, State-Ease Inc., Minneapolis, MN, USA) software package was used to analyze the experimental data. *P*-values of less than 0.05 were considered to be statistically significant.

2.4. Total phenolics determination

Total phenolic content was determined using Folin-Ciocalteu reagent and gallic acid as the standard (Lin & Tang, 2007). The content of total phenols was calculated on the basis of the calibration curve of gallic acid and expressed as g/100 g of a dry weight of SBP.

2.5. Superoxide anion radical scavenging activity

Superoxide radicals were generated by pyrogallic acid method (Jiang, Zhang, Liu, Wang, & Fan, 2010), and the method was innovated slightly. The system contained 2.5 mL of PBS buffer (0.1 M, pH 8.2), 4 mL of sample solution, 2.5 mL of pyrogallic acid (6.0 mM), and 0.5 mL of thick hydrochloric acid for termination the reaction. The solution was incubated at 25 °C and determined at 299 nm. Vc was used as a reference material. All tests were performed in triplicate. The scavenging activity was calculated as follows:

$$\text{scavenging activity (\%)} = \frac{A_0 - (A_s - A_c)}{A_0} \times 100$$

where A_s, with the presence of pyrogallic acid and test polysaccharides; A_0, with the presence of pyrogallic acid but without test polysaccharides; and A_c, with the presence of test polysaccharides but without pyrogallic acid.

2.6. Radical scavenging activities on DPPH

The free radical scavenging activity of the prepared polysaccharides was measured using DPPH by the method of Blois

(1958). A 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of various concentrations (0.2–1.0 mg/mL) of sample dissolved in methanol to be tested. After 30 min, absorbance was measured at 517 nm. Vc was used as a reference material. All tests were performed in triplicate. The scavenging activity was calculated as follows:

$$\text{scavenging activity}\,(\%) = \frac{A_0 - (A_s - A_c)}{A_0} \times 100$$

where A_s is the absorbance of the test sample mixed with DPPH solution (3 mL sample + 1 mL DPPH); A_0 is the absorbance of DPPH solution without sample (1 mL DPPH + 3 mL methanol) and A_c, is the absorbance of the sample without DPPH solution (3 mL sample + 1 mL methanol).

2.7. Hydroxyl radical-scavenging activity

Hydroxyl radical-scavenging activity was measured according to Smirnoff's work (Smirnoff & Cumbes, 1989). 0.5 mL $FeSO_4$ (1.5 mM) was mixed with 0.35 mL H_2O_2 (6 mM), 0.15 mL sodium salicylate (20 mM) and 1 mL sample (0.2-1.0 mg/mL), then incubated for 1 h at 37 °C. The absorbance of the hydroxylated salicylate complex was measured at 562 nm. Ascorbic acid was used as the positive control. The antioxidant activity was calculated with the following equation:

$$\text{scavenging effect}\,(\%) = \frac{1 - (A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}} \times 100$$

where A_{sample} was the absorbance of the test (sample or ascorbic acid), A_{control} was the absorbance of the solvent control, and A_{blank} was the absorbance of the reagent blank without sodium salicylate.

2.8. Statistical analysis

Data are reported as the mean ± SD of three measurements. The scientific statistic software GraphPad Prism 3.03 was used to evaluate the significance of differences between groups. Comparisons between groups were done using Kruskal–Wallis test followed by Dunn's post hoc test. $P < 0.05$ was regarded as significant. The IC_{50} (the concentration of antioxidant at which 50% of the reaction was inhibited) was determined using the statistics program SPSS for Windows, version 13.0.

3. Results and discussion

3.1. Statistical analysis and the model fitting

The effects of four process variables (i.e. temperature ($X1$), time ($X2$), number ($X3$) and ratio of water to raw material ($X4$)) were studied during experimentation. The results of 27 runs using BBD design are presented in Table 2 that include the design, experimental values and the predicted values, where the predicted values of the responses were obtained from quadratic model fitting techniques by the software mentioned above. BBD with four factors and three levels, including three replicates at the center point, was used to fit a second-order response surface in order to optimize the extraction conditions. The three center point runs were carried out to measure the process stability and inherent variability. Maximum extraction yield of SBP (2.09%) was recorded under the experimental conditions of extraction temperature of 70 °C, extraction time of 2 h, number of extraction of 3, and ratio of water to raw material of 20. The polysaccharides yield was taken as the response Y. Predicted responses Y could be expressed by the following second order polynomial equations:

Table 2
BBD with the experimental values and predicted values for extraction yield of SBP.

No.	X_1	X_2	X_3	X_4	Extraction yield (%)	Predicted yield (%)
1	−1	−1	0	0	0.36	0.33
2	1	−1	0	0	1.75	1.60
3	−1	1	0	0	0.44	0.54
4	1	1	0	0	2.10	2.07
5	0	0	−1	−1	0.57	0.55
6	0	0	1	−1	1.27	1.20
7	0	0	−1	1	0.74	0.76
8	0	0	1	1	1.15	1.12
9	−1	0	0	−1	0.37	0.34
10	1	0	0	−1	1.65	1.71
11	−1	0	0	1	0.39	0.38
12	1	0	0	1	1.73	1.81
13	0	−1	−1	0	0.54	0.67
14	0	1	−1	0	0.88	0.79
15	0	−1	1	0	0.83	0.96
16	0	1	1	0	1.61	1.52
17	−1	0	−1	0	0.28	0.24
18	1	0	−1	0	1.53	1.53
19	−1	0	1	0	0.62	0.63
20	1	0	1	0	2.09	2.14
21	0	−1	0	−1	0.68	0.67
22	0	1	0	−1	0.99	1.07
23	0	−1	0	1	0.88	0.80
24	0	1	0	1	1.06	1.08
25	0	0	0	0	1.03	1.05
26	0	0	0	0	1.05	1.05
27	0	0	0	0	1.06	1.05

$$\begin{aligned} Y = {} & 1.05 + 0.70 \times X_1 + 0.17 \times X_2 + 0.25 \times X_3 + 0.035 \times X_4 \\ & + 0.067 \times X_1 \times X_2 + 0.055 \times X_1 \times X_3 + 0.015 \times X_1 \times X_4 \\ & + 0.11 \times X_2 \times X_3 - 0.032 \times X_2 \times X_4 - 0.073 \times X_3 \times X_4 + 0.12 \\ & \times X_1 \times X_1 - 0.03 \times X_2 \times X_2 - 0.031 \times X_3 \times X_3 - 0.11 \times X_4 \times X_4 \end{aligned} \quad (2)$$

where X_1 denotes extraction temperature, °C; X_2 denotes extraction time, h; X_3 denotes number of extraction; X_4 denotes ratio of water to raw material, mL/g.

The analysis of variance (ANOVA) result of the model is shown in Table 3 including a good model performance with the correlation coefficient (R^2) value of 0.9838. The calculated model was able to explain 98.38% of the result in the case of the polysaccharides extraction ratio. Generally, exploration and optimization fitted response surface may mislead results, unless the model exhibits a good fit, which makes checking the model adequacy essential (Wang, Yang, Du, & Yi, 2008). The statistical analysis gave high significant level ($P < 0.0001$), attesting the goodness of fit of the model in case of the polysaccharides extraction rate. The value of coefficient of variation (C.V.) was 9.78%, suggested that the model is reproducible (Wanasundara & Shahidi, 1996). The results indicated that the model could work well for the prediction of polysaccharides extract from *S. barbata*.

3.2. Optimization of extraction conditions of SBP

The best way of expressing the effect of any independent variables on the polysaccharides extraction yield is to generate surface response plots of the model, which were done by varying two variables within the experimental range under investigation and holding the other two variables at its '0' level. The 3D plot in Fig. 1a showed the effects of extraction temperature (X_1) and extraction time (X_2) on polysaccharides yield (Y). There was a rapid rise in extraction yield with increase in extraction temperature

Table 3
Estimated regression coefficients for the quadratic polynomial model and the analysis of variance (ANOVA) for the experimental results.

Parameter[a]	Estimated coefficients	Standard error	DF[b]	Sum of squares	*F*-value	Prob > *F*
Intercept						
α_0	1.05	0.058	1			
Linear						
X_1	0.70	0.029	1	5.87	584.85	<0.0001
X_2	0.17	0.029	1	0.35	34.58	<0.0001
X_3	0.25	0.029	1	0.77	76.28	<0.0001
X_4	0.035	0.029	1	0.015	1.47	<0.0001
Quadratic						
$X_1 \times X_1$	0.12	0.043	1	0.076	7.55	0.0177
$X_2 \times X_2$	−0.030	0.043	1	4.668E−003	0.47	0.5081
$X_3 \times X_3$	−0.031	0.043	1	5.070E−003	0.51	0.4907
$X_4 \times X_4$	−0.11	0.043	1	0.064	6.39	0.0266
Interaction						
$X_1 \times X_2$	0.067	0.050	1	0.018	1.82	0.2026
$X_1 \times X_3$	0.055	0.050	1	0.012	1.21	0.2936
$X_1 \times X_4$	0.015	0.050	1	9.000E−004	0.090	0.7696
$X_2 \times X_3$	0.11	0.050	1	0.048	4.83	0.0484
$X_2 \times X_4$	−0.032	0.050	1	4.225E−003	0.42	0.5285
$X_3 \times X_4$	−0.073	0.050	1	0.021	2.10	0.1733
Model			14	7.32	52.15	<0.0001
Lack of fit			10	0.12	51.38	0.0192
Pure error			2	4.667E−004		
R^2	0.9838		Adjusted R^2	0.9649		
C.V.%	9.78					

[a] Coefficients refer to the general model.
[b] Degree of freedom.

(X_1); however, extraction yield (Y) was found to rise slightly with extraction time (X_2) varying from 1 to 3 h. In Fig. 1b, there was an upsurge in extraction yield (Y) with increase in extraction temperature (X_1), but extraction yield (Y) showed a slight escalating trend with increase in number of extraction (X_3). Similarly, Fig. 1c shows that extraction yield (Y) soared rapidly with increase in extraction temperature (X_1), but rose gently with increase in ratio of water to raw material (X_4). Interestingly, Fig. 1a–c commonly demonstrates that extraction temperature was the major factor among the four factors causing significant effects on extraction yield. This finding was consistent with the previous literatures on polysaccharides (Hou, Lin, Lu, Fang, & Chen, 2006; Jiang, Qiao, & Zhang, 2005). Polysaccharides yield (Y) affected by varying extraction time (X_2) and number of extraction (X_3) was shown in Fig. 1d. It could be seen that contributions of extraction time (X_2) and number of extraction (X_3) to the effects on extraction yield (Y) were almost similar, demonstrating that extraction time (X_2) had a similar effect on extraction yield (Y) as number of extraction (X_3). Fig. 1e indicates that extraction time (X_2) was the major factor affecting extraction yield (Y). The longer extraction time (X_2), the higher extraction yield (Y). Nevertheless, extraction yield (Y) was found to rise slightly with ratio of water to raw material (X_4) varying from 15 to 25. In a trend similar to Fig. 1e and f shows that the increase in number of extraction (X_3) led to a rapid rise to extraction yield (Y), whereas extraction yield (Y) showed a slight escalating trend with increase in ratio of water to raw material (X_4).

3.3. Verification of predictive model

The suitability of the model equations for predicting optimum response values was tested under the conditions: extraction temperature of 70 °C, extraction time of 3 h, number of extraction of 3, and ratio of water to raw material of 18.5 mL/g. This set of conditions was determined to be optimum by the RSM optimization approach and was also used to validate experimentally and predict the values of the responses using the model equation. A mean value of 2.43 ± 0.11% (n = 3), obtained from real experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process (Table 4).

3.4. Total phenolic content in SBP

The antioxidant activity except of polysaccharides is responsible phenolics as well. Therefore, the phenolic content in polysaccharides sample was determined. The total phenol content of SBP was 0.3 ± 0.1 g/100 g of a dry weight of SBP (n = 3). The result indicated that the phenolic content in SBP was relatively low and the antioxidant activity of SBP mainly resulted from polysaccharides.

3.5. Superoxide anion radical scavenging activity

The superoxide radical is a highly toxic species can be generated by numerous biological and photochemical reactions. In addition to directly attacking important biological molecules, superoxide radical may also decompose to form singlet oxygen and hydroxyl radicals, which may increase local oxidative stress and initiate cellular damage or lipid peroxidation and pathological incidents such as arthritis and Alzheimer's disease (Liu, Wang, Pang, Yao, & Gao, 2010).

As shown in Fig. 2, superoxide scavenging activities of SBP and ascorbic acid increased with increasing concentrations, and the superoxide radical scavenging rate of SBP and ascorbic acid at 1.0 mg/mL was 67.1% and 90.2%, respectively. The IC_{50} value of SBP and ascorbic acid for eliminating superoxide radicals was about 0.17 and 0.19 mg/mL, respectively, which indicated that both the polysaccharides and ascorbic acid had significant superoxide radical scavenging activity.

3.6. DPPH radical scavenging activity

DPPH is one of the compounds that possessed a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavenger (Song, Zhang, Zhang, & Wang, 2010). On interacting with DPPH, antioxidants transfer

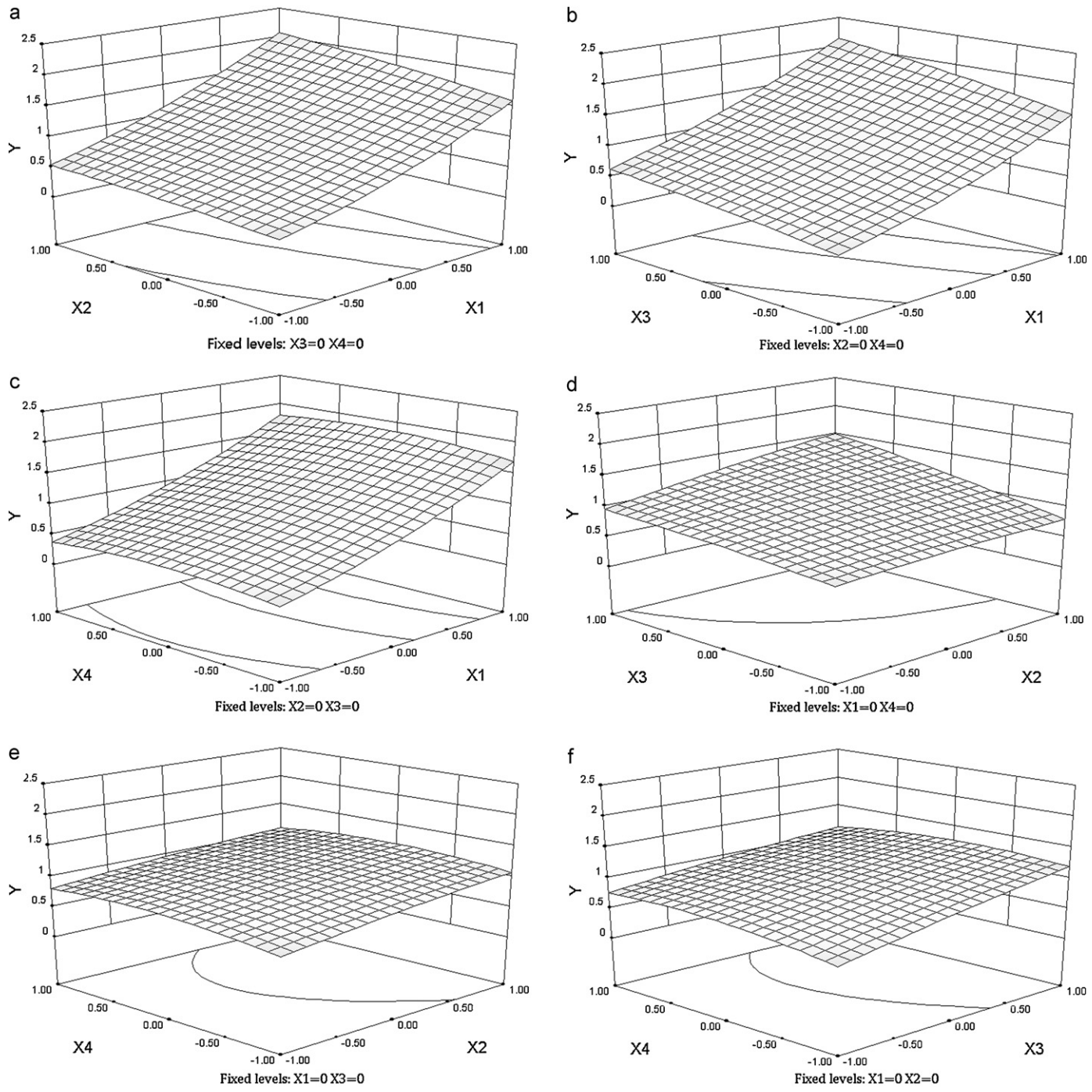

Fig. 1. Response surface (3D) showing the effect of the extraction temperature, extraction time, number of extraction and ratio of water to raw material on the response *Y*.

either an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character (Naik et al., 2003).

The result of DPPH free radical-scavenging ability of the polysaccharides is shown in Fig. 3 and compared with ascorbic acid as control standards. As can be seen from Fig. 3, the DPPH radical scavenging increased from 28.4% to 60.3%, when the concentration of the polysaccharides increased from 0.2 to 1.0 mg/mL. The IC_{50} of the polysaccharide and Vc were 0.57 and

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

Optimum condition				Extraction yield of SBP (%)	
Extraction temperature	Extraction time	Extraction number	Ratio of water to raw material	Experimental[a]	Predicted
70 °C	3 h	3	18.5 mL/g	2.43 ± 0.11	2.47

[a] Mean ± standard deviation (n = 3).

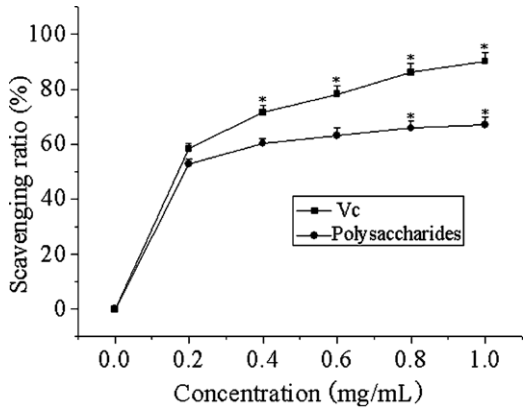

Fig. 2. Inhibition effects of the polysaccharides and ascorbic acid (Vc) on superoxide radical. Data are mean ± SD for three measurements. *$P < 0.05$ compared with control (% inhibition by control = 0%; Kruskal–Wallis test followed by Dunn's post hoc test).

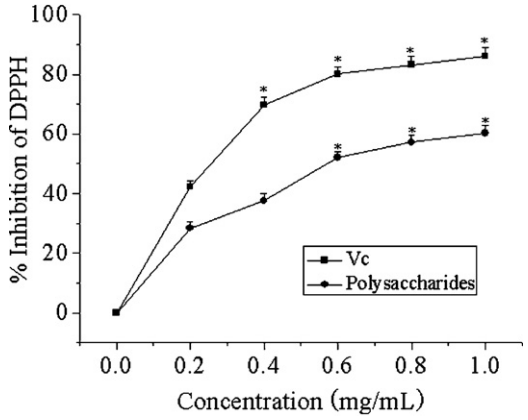

Fig. 3. Inhibition effects of the polysaccharides and ascorbic acid (Vc) on DPPH radical. Data are mean ± SD for three measurements. *$P < 0.05$ compared with control (% inhibition by control = 0%; Kruskal–Wallis test followed by Dunn's post hoc test).

0.26 mg/mL, respectively. The scavenging ability was lower than that of ascorbic acid. Similar results have been reported in other plant polysaccharides (Yuan, Zhang, Fan, & Yang, 2008; Zha et al., 2009).

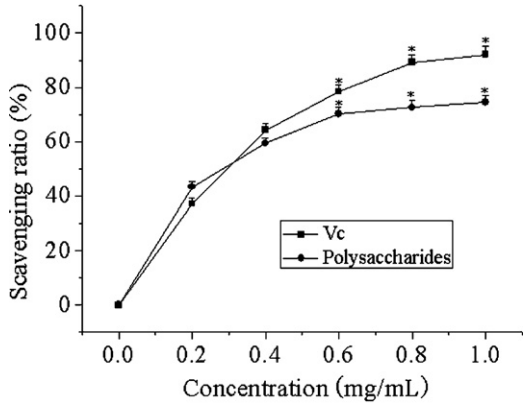

Fig. 4. Inhibition effects of the polysaccharides and ascorbic acid (Vc) on hydroxyl radical. Data are mean ± SD for three measurements. *$P < 0.05$ compared with control (% inhibition by control = 0%; Kruskal–Wallis test followed by Dunn's post hoc test).

3.7. Scavenging activity on hydroxyl radical

Except for superoxide radical, hydroxyl radical is considered to be a highly potent oxidant, which can react with all biomacromolecules functioning in living cells (Gülçin, 2006). The result of hydroxyl free radical-scavenging ability of the polysaccharides is shown in Fig. 4 and compared with that of ascorbic acid. The hydroxyl radical scavenging rate of SBP and ascorbic acid at 1.0 mg/mL was 74.5% and 92.1%, respectively. The IC_{50} value of SBP and ascorbic acid for eliminating hydroxyl radicals was about 0.3 and 0.28 mg/mL, respectively, which indicated that the scavenging activity of SBP against hydroxyl radical was slightly higher than that of ascorbic acid.

4. Conclusion

The extraction conditions have significant effects on the yield of SBP. Using the surface plots in RSM was effective for estimating the effect of four independent variables (extraction temperature, °C; extraction time, h; number of extraction and ratio of water to raw material, mL/g). The optimum parameters were: extraction temperature 70 °C, extraction time 3 h, number of extraction 3 and ratio of water to raw material 18.5 mL/g. This set of optimum parameters gives maximum predicted value of the responses (polysaccharide yield 2.47%). Meanwhile, under these conditions, the mean experimental value (polysaccharide yield 2.43 ± 0.11%) corresponded well with the predicted value. Additionally, the results of scavenging activity showed that the polysaccharides had significant antioxidant activity and free radical-scavenging activity. The free radical-scavenging property may be one of the mechanisms by which this drug is useful as a health food as well as a traditional Chinese medicine. Further investigation of its antioxidant activities in vivo and antioxidant mechanisms will be performed in future studies.

References

Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. *Nature*, *181*, 1199–1200.
Borkowski, J. J. (1995). Spherical prediction-variance properties of central composite and Box–Behnken designs. *Technometrics*, *37*, 399–410.
Capek, P., Machová, E., & Turjan, J. (2009). Scavenging and antioxidant activities of immunomodulating polysaccharides isolated from *Salvia officinalis* L. *International Journal of Biological Macromolecules*, *44*, 75–80.
Chen, J., Zhang, T., Jiang, B., Mu, W., & Miao, M. (2012). Characterization and antioxidant activity of Ginkgo biloba exocarp polysaccharides. *Carbohydrate Polymers*, *87*, 40–45.
Gan, C. Y., Abdul Manaf, N., & Latiff, A. A. (2010). Optimization of alcohol insoluble polysaccharides (AIPS) extraction from the *Parkia speciosa* pod using response surface methodology (RSM). *Carbohydrate Polymers*, *79*, 825–831.
Gan, C. Y., & Latiff, A. A. (2011). Extraction of antioxidant pectic-polysaccharide from mangosteen (*Garcinia mangostana*) rind: Optimization using response surface methodology. *Carbohydrate Polymers*, *83*, 600–607.
Giovanni, M. (1983). Response surface methodology and product optimization. *Food Technology*, *3741*, 45.
Gülçin, İ. (2006). Antioxidant and antiradical activities of L-carnitine. *Life Sciences*, *78*, 803–811.
Guyton, K. Z., Bhan, P., Kuppusamy, P., Zweier, J. L., Trush, M. A., & Kensler, T. W. (1991). Free radical-derived quinone methide mediates skin tumor promotion by butylated hydroxytoluene hydroperoxide: Expanded role for electrophiles in multistage carcinogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *88*, 946–950.
Hou, S. J., Lin, C. Q., Lu, J., Fang, H. M., & Chen, C. G. (2006). Study on technology of extracting polysaccharides from *polygonatum* with water. *Anhui Chemical Industry*, *142*, 20–22 (in Chinese).
Hu, X., You, J., Bao, C., Zhang, H., Meng, X., Xiao, T., et al. (2008). Determination of total flavonoids in *Scutellaria barbata* D. Don by dynamic ultrasonic extraction coupled with on-line spectrophotometry. *Analytica Chimica Acta*, *610*, 217–223.
Jiang, Q., Qiao, X., & Zhang, Z. (2005). Effect of extraction conditions on the yield of neutral polysaccharide from garlic (*Allillm sativum* L.). *Journal of Chinese Institute of Food Science and Technology*, *5*, 101–105 (in Chinese).
Jiang, B., Zhang, H., Liu, C., Wang, Y., & Fan, S. (2010). Extraction of water-soluble polysaccharide and the antioxidant activity from *Ginkgo biloba* leaves. *Medicinal Chemistry Research*, *19*, 262–270.

Karacabey, E., & Mazza, G. (2010). Optimisation of antioxidant activity of grape cane extracts using response surface methodology. *Food Chemistry*, *119*, 343–348.
Kil, H. Y., Seong, E. S., Ghimire, B. K., Chung, I. M., Kwon, S. S., Goh, E. J., et al. (2009). Antioxidant and antimicrobial activities of crude sorghum extract. *Food Chemistry*, *115*, 1234–1239.
Kim, D. I., Lee, T. K., Lim, I. S., Kim, H., Lee, Y. C., & Kim, C. H. (2005). Regulation of IGF-I production and proliferation of human leiomyomal smooth muscle cells by *Scutellaria barbata* D. Don in vitro: Isolation of flavonoids of apigenin and luteolon as acting compounds. *Toxicology and Applied Pharmacology*, *205*, 213–224.
Kimmel, C. A., Kimmel, G. L., & Frankos, V. (1986). Interagency Regulatory Liaison Group Workshop on reproductive toxicity risk assessment. *Environmental Health Perspectives*, *66*, 193–221.
Kinsella, J. E., Frankel, E. N., German, J. B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology*, *47*, 85–89.
Lee, T. K., Lee, D. K., Kim, D. I., Lee, Y. C., Chang, Y. C., & Kim, C. H. (2004). Inhibitory effects of *Scutellaria barbata* D. Don on human uterine leiomyomal smooth muscle cell proliferation through cell cycle analysis. *International Immunopharmacology*, *4*, 447–454.
Lin, J. Y., & Tang, C. Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chemistry*, *101*, 140–147.
Liu, W., Wang, H., Pang, X., Yao, W., & Gao, X. (2010). Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*. *International Journal of Biological Macromolecules*, *46*, 451–457.
Lu, C. H., Engelmann, N. J., Lila, M. A., & Erdman, J. W., Jr. (2008). Optimization of lycopene extraction from tomato cell suspension culture by response surface methodology. *Journal of Agricultural and Food Chemistry*, *56*, 7710–7714.
Luo, A. X., He, X. J., Zhou, S. D., Fan, Y. J., Luo, A. S., & Chun, Z. (2010). Purification, composition analysis and antioxidant activity of the polysaccharides from *Dendrobium nobile* Lindl. *Carbohydrate Polymers*, *79*, 1014–1019.
Naik, G. H., Priyadarsini, K. I., Satav, J. G., Banavalikar, M. M., Sohoni, D. P., Biyani, M. K., et al. (2003). Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. *Phytochemistry*, *63*, 97–104.
Nandita, S., & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, *85*, 611–616.
Paucar-Menacho, L. M., Berhow, M. A., Mandarino, J. M. G., de Mejia, E. G., & Chang, Y. K. (2010). Optimisation of germination time and temperature on the concentration of bioactive compounds in Brazilian soybean cultivar BRS 133 using response surface methodology. *Food Chemistry*, *119*, 636–642.
Ravikumar, K., Pakshirajan, K., Swaminathan, T., & Balu, K. (2005). Optimization of batch process parameters using response surface methodology for dye removal by a novel adsorbent. *Chemical Engineering Journal*, *105*, 131–138.
Rugo, H., Shtivelman, E., Perez, A., Vogel, C., Franco, S., Melisko, M., et al. (2007). Phase I trial and antitumor effects of BZL101 for patients with advanced breast cancer. *Breast Cancer Research and Treatment*, *105*, 17–28.
Sahreen, S., Khan, M. R., & Khan, R. A. (2010). Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food Chemistry*, *122*, 1205–1211.
Sato, Y., Suzaki, S., Nishikawa, T., Kihara, M., Shibata, H., & Higuti, T. (2000). Phytochemical flavones isolated from *Scutellaria barbata* and antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology*, *72*, 483–488.
Shi, R., Qiao, S., Yu, D., Shi, X., Liu, M., Jiang, X., et al. (2011). Simultaneous determination of five flavonoids from *Scutellaria Barbata* extract in rat plasma by LC–MS/MS and its application to pharmacokinetic study. *Journal of Chromatography B*, *879*, 1625–1632.
Smirnoff, N., & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, *28*, 1057–1060.
Song, H., Zhang, Q., Zhang, Z., & Wang, J. (2010). In vitro antioxidant activity of polysaccharides extracted from *Bryopsis plumosa*. *Carbohydrate Polymers*, *80*, 1057–1061.
Suh, S. J., Yoon, J. W., Lee, T. K., Jin, U. H., Kim, S. L., Kim, M. S., et al. (2007). Chemoprevention of *Scutellaria bardata* on human cancer cells and tumorigenesis in skin cancer. *Phytotherapy Research*, *21*, 135–141.
Sun, Y., Liu, J., & Kennedy, J. F. (2010). Application of response surface methodology for optimization of polysaccharides production parameters from the roots of *Codonopsis pilosula* by a central composite design. *Carbohydrate Polymers*, *80*, 949–953.
Wanasundara, P. K. J. P. D., & Shahidi, F. (1996). Optimization of hexametaphosphate-assisted extraction of flaxseed proteins using response surface methodology. *Journal of Food Science*, *61*, 604–607.
Wang, P., Geng, X., Pi, G., Shi, R., Yuan, Z., & Wang, C. (2010). Preparative separation of four individual flavonoids in *Scutellaria barbata* D. Don based on high selectivity polymeric adsorbents with different polarities. *Journal of Chromatography B*, *878*, 3375–3381.
Wang, J., Sun, B. G., Cao, Y. P., Tian, Y., & Li, X. L. (2008). Optimization of ultrasound assisted extraction of phenolic compounds from wheat bran. *Food Chemistry*, *106*, 804–810.
Wang, L. Z., Yang, B., Du, X. Q., & Yi, C. (2008). Optimisation of supercritical fluid extraction of flavonoids from *Pueraria lobata*. *Food Chemistry*, *108*, 737–741.
White, C., Willis, B., Keshav, N., & Dutton, S. (2001). Identifying and estimating significant geologic parameters with experimental design. *SPE Journal*, *6*, 311–324.
Wong, B. Y., Lau, B. H., Yamasaki, T., & Teel, R. W. (1993). Modulation of cytochrome P-450IA1-mediated mutagenicity, DNA binding and metabolism of benzo[a]pyrene by Chinese medicinal herbs. *Cancer Letters*, *68*, 75–82.
Yang, N. L., Qu, H. B., & Cheng, Y. Y. (2004). An optimization method for extraction process of Copt-Chinensis with uniform design and regression analysis. *Journal of Chemical Engineering of Chinese Universities*, *18*, 126–130 (in Chinese).
Yuan, J. F., Zhang, Z. Q., Fan, Z. C., & Yang, J. X. (2008). Antioxidant effects and cytotoxicity of three purified polysaccharides from *Ligusticum chuanxiong* Hort. *Carbohydrate Polymers*, *74*(4), 822–827.
Zha, X. Q., Wang, J. H., Yang, X. F., Liang, H., Zhao, L. L., Bao, S. H., et al. (2009). Antioxidant properties of polysaccharide fractions with different molecular mass extracted with hot-water from rice bran. *Carbohydrate Polymers*, *78*, 570–575.
Zheng, Y., Wei, X., & Long, J. (2010). Advances in studies on Scutellariae Barbatae Herba. *Chinese Traditional and Herbal Drugs*, *41*, 1406–1408 (in Chinese).
Zhong, K., & Wang, Q. (2009). Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. *Carbohydrate Polymers*, *80*, 19–25.
Zykwinska, A., Rondeau-Mouro, C., Garnier, C., Thibault, J. F., & Ralet, M. C. (2006). Alkaline extractability of pectic arabinan and galactan and their mobility in sugar beet and potato cell walls. *Carbohydrate Polymers*, *65*, 510–520.