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Characterization and antioxidant activity of a polysaccharide extracted from *Sarcandra glabra*

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ABSTRACT

Response surface methodology (RSM) was employed to optimize the parameters for polysaccharide extracted from the aerial parts of *Sarcandra glabra* (SGP). The optimum conditions were predicted as follows, ratio of water to raw material at 30, extracting temperature at 85 °C, extracting duration at 3 h, and yield was estimated at 4.55%. The experimental yield of SGP under the optimum conditions was 4.49 ± 0.09%. A homogenous polysaccharide (SGP-1) was obtained by purification using DEAE-cellulose-52 and Sephacryl S-400 column chromatography. SGP-1 showed a single symmetrical peak in high performance size-exclusion chromatography (HPSEC) and the average molecular weight (Mw) was estimated to be 1.06×10^4 Da. It was composed of glucose, galactose, and mannose in a ratio of 8.38:3.13:1 determined by gas chromatography (GC). The *in vitro* antioxidant tests showed that SGP-1 has significant inhibition effects on hydroxyl, superoxide anion, DPPH, ABTS radicals in a dose-dependent manner. This study indicated that SGP-1 could be used as a potential natural antioxidant.

1. Introduction

Sarcandra glabra (Thunb.) Nakai (Chinese name: Zhongjiefeng or Caoshanhu) is considered as an important herb in traditional Chinese medicines (TCMs). Medical components prepared from this herb are used as antitumor or anti-inflammatory drugs in China (Zheng, Wang, Chen, & Hu, 2003). Commercials like toothpaste, health tea, cosmetics, and chewing gum made from this plant are also bestselling. *S. glabra* is mainly distributed in the southern of China and Southeast of Asia. It is estimated that about 100 million kilograms materials of *S. glabra* are required in 5 years to satisfy the huge market (Cai & Chen, 2010). Until now, most of the researches were focus on isolation and activity of flavonoids, coumarins, triterpenoids, sesquiterpenes, and other phenolic components of this plant (Feng et al., 2010; He et al., 2011; Xiao, Guo, Deng, & Li, 2009), but little attention was devoted to the extraction and function of biomacromolecules (especially polysaccharides) from *S. glabra*.

Polysaccharides from natural sources are considered to be effective, non-toxic substances. Many polysaccharides could be explored into drugs for their pharmacological activities. Antioxidants are helpful to alleviate stress-induced diseases including inflammatory, cardiac disorders, diabetes mellitus, and neurodegenerative diseases (Ananthi et al., 2010). Recently, several reports indicated some polysaccharides extracted from botanicals and fungus could be explored as potential antioxidants (Hua et al., 2012; Jin, 2012). Hot water extraction is a classical polysaccharide extraction method. In the process of hot water extraction, the crude polysaccharide yield was depended on the parameters as follows, solid-to-liquid ratio, extraction temperature, duration, and extraction times. Optimization of extraction parameters is important for further study of the polysaccharide from *S. glabra*.

The present study was conducted firstly to optimize extracting parameters using response surface methodology (RSM). After isolation and purification, preliminary characterization of the homogenous polysaccharide (SGP-1) was performed. In addition, the antioxidant activity of SGP-1 was estimated through a series of tests, including hydroxyl, superoxide anion, DPPH, ABTS radical scavenging, ferrous ion chelating, and reducing power determinations.

2. Materials and methods

2.1. Materials and chemicals

The materials of *S. glabra* were purchased from Nanchang, Jiangxi Province, China, in October 2010. The diethylaminoethyl-cellulose (DEAE-52), Sephacryl S-400 for chromatography and dextran standards of T-70, T-40, T-20, T-10, and T-5 were obtained from Pharmacia Co. Ltd. (Uppsala, Sweden). Thirty percent hydrogen peroxide was purchased from Sinopharm

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Table 1
Box–Behnken experimental design and results for extraction yield.

Run	Factor 1 A: water to solid	Factor 2 B: temperature	Factor 3 C: extract duration	Experimental yield (%)
1	25(0)	90(1)	3(1)	4.27 ± 0.06
2	20(−1)	80(0)	3(1)	3.91 ± 0.05
3	25(0)	80(0)	2(0)	4.20 ± 0.09
4	25(0)	80(0)	2(0)	4.28 ± 0.06
5	25(0)	70(−1)	1(−1)	3.54 ± 0.07
6	25(0)	70(−1)	3(1)	3.75 ± 0.11
7	30(1)	80(0)	1(−1)	3.79 ± 0.03
8	20(−1)	90(1)	2(0)	3.88 ± 0.09
9	30(1)	70(−1)	2(0)	3.54 ± 0.04
10	25(0)	90(1)	1(−1)	3.65 ± 0.11
11	25(0)	80(0)	2(0)	4.35 ± 0.05
12	25(0)	80(0)	2(0)	4.34 ± 0.01
13	20(−1)	70(−1)	2(0)	3.64 ± 0.13
14	30(1)	90(1)	2(0)	4.15 ± 0.10
15	20(−1)	80(0)	1(−1)	3.65 ± 0.13
16	30(1)	80(0)	3(1)	4.53 ± 0.07
17	25(0)	80(0)	2(0)	4.37 ± 0.04

chemical reagent Co. Ltd. D-Glucose (Glc), D-galactose (Gal), D-mannose (Man), L-rhamnose (Rha), D-xylose (Xyl), D-arabinose (Ara), Gallic acid, trichloride ferric ($FeCl_3$), ferrous sulfate ($FeSO_4$), ethylene diamine tetra-acetic acid (EDTA), ferrozine, nitroblue tetrazolium (NBT), Tris–HCl buffer, thiobarbituric acid (TBA), 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), 2-2-azino-bis-(3-ethyl-benzthia-zoline-6-sulfonic acid) (ABTS), and ascorbic acid (Vc) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trifluoroacetic acid (TFA) was from Merck (Germany). All other reagents of analytical grade were purchased from Shanghai Chemical Co. (Shanghai, China).

2.2. Extraction method

5 kg aerial parts of *S. glabra* was crushed to pass through 80 mesh sieve and refluxed with 90% ethanol at 80 °C in water bath for 3 h to remove fats, pigments, and some oligosaccharides. Each dried pretreated sample (50 g) was extracted with hot water in designed conditions. The extraction solutions were collected by centrifugation (4000 r/min, 5 min). Subsequently, aqueous extract was concentrated to 1/3 of the original volume, cooled, and precipitated with four volumes of absolute ethanol for overnight at 4 °C. The precipitates collected by centrifugation (4000 r/min, 5 min) were washed three times with absolute ethanol and dried under reduced pressure. Thus, the crude polysaccharide named SGP was obtained for follow-up study. The yields (%) of crude polysaccharide in the extraction were calculated by the following equations:

$$\text{Polysaccharides yield \% (w/w)} = \frac{\text{dried crude extraction weight}}{\text{powder weight (50 g)}} \times 100\% \quad (1)$$

2.3. Experimental design

On the basis of single-factor experiment test (data were not shown), a Box–Behnken design (BBD) with three independent variables (X_1, ratio of water to raw material, X_2, extraction temperature, X_3, extraction duration) at three levels were performed. For statistical calculation, the variables were coded by the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (2)$$

where x_i was the independent variable coded value, X_i was the independent variable real value, X_0 was the independent variable real value on the center point and ΔX_i was the step change value. The range of independent variables, a BBD matrix and the response values carried out for developing the model were listed in Table 1.

The whole designed experiment consisted of 17 trial points in a random order. These trials were divided into 12 factorial points, and 5 replicates which were used for the estimation of a pure error sum of squares at the center of the design. The response value in each trial was an average of duplicates. Based on the BBD experimental data, regression analysis was carried out and fitted into the empirical quadratic second-order polynomial model:

$$Y = \sum A_0 + \sum_{i=1}^{3} A_i X_i \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j \quad (3)$$

where Y represented the response function, A_0, A_i, A_{ii}, A_{ij} were the regression coefficients of variables for intercept, linear, quadratic, and interaction terms respectively, and X_i, X_j were levels of the independent variables ($i \neq j$). The coefficients of the second polynomial model and the response surfaces obtained from the experimental design were fitted to multiple nonlinear regressions using software Design-Expert 7.1.3 (Stat-Ease, Inc., USA). The fitness of the polynomial model equation was represented by the coefficient of determination R^2, the statistical significance was checked by F-test at a probability (P-value) of 0.01 or 0.05. The significances of the regression coefficients were also tested by F-test.

2.4. Purification of polysaccharide

The crude polysaccharide extracted from *S. glabra* (0.5 g) was dissolved in deionized water, centrifuged, and then the supernatant was applied to a DEAE-cellulose-52 column (2.6 cm × 30 cm) equilibrated with deionized water. Elution was carried out with deionized water, subsequently with a linear gradient of 0–2 M NaCl aqueous solutions at a flow rate of 18 mL/h. Each fraction in test tube (3 mL) was collected by an automated step-by-step fraction collector, and monitored by the phenol–sulfuric acid method at 490 nm. The deionized water fraction was then applied to a Sephacryl S-400 column (1.6 cm × 100 cm) equilibrated and eluted with deionized water at room temperature at a flow rate of 18 mL/h. Fractions were collected and analyzed by phenol–sulfuric acid method at 490 nm. The homogeneous fractions were gathered, concentrated, and lyophilized to give a white powdery polysaccharide which was coded as SGP-1. The total carbohydrate content of SGP-1 was determined by phenol–sulfuric acid method with D-glucose

as standard at 490 nm (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.5. Molecular weight determination

The average molecular weight of SGP-1 was determined by high performance size-exclusion chromatography (HPSEC), which was performed on an Agilent 1100 HPLC system equipped with a Shodex SUGAR KS-805 column (8.0 mm ID × 300 mm) and a RID detector. The mobile phase was deionized water, and the flow rate was 1.0 mL/min at 30 °C. 1 mg sample was dissolved in the mobile phase (1.0 mL), and 20 μL of supernatant was injected after centrifuged (8000 rpm; 3 min). The molecular mass was estimated by the comparison to a calibration curve prepared with the T-series dextran standards of known molecular masses (T-70, T-40, T-20, T-10, and T-5).

2.6. Analysis of monosaccharide compositions

SGP (5 mg) was hydrolyzed in 1 mL of 2 M TFA at 100 °C for 8 h. The product was reduced with $NaBH_4$ at 65 °C for 1 h, acetylated with mixture of pyridine and acetic anhydride (1:1, v/v) at 100 °C for 1 h, and then analyzed by gas chromatography (GC) with a HP-5 capillary column (HP6820, Hewlett–Packard). The temperature of the column was held at 150 °C for 2 min, increased to 220 °C at a rate of 2 °C/min, and sequentially increased to 280 °C at a rate of 30 °C/min with N_2 as the carrier gas and inositol as the internal standard. Standard monosaccharides (L-rhamnose, D-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose) were also derived and analyzed under the same procedure as references (Honda, Suzuki, Kakehi, Honda, & Takai, 1981).

2.7. FT-IR spectroscopy

The FT-IR spectrum of SGP-1 was recorded with a Nicolet 5700 IR spectrometer with the range of 4000–400 cm^{-1}. The sample was analyzed as KBr pellets.

2.8. Total phenolics determination

Total phenolics content of SGP-1 was determined by the method (Khokhar & Magnusdottir, 2002) using Folin-Ciocalteu reagent, using gallic acid as the standard. The content of total phenolics was calculated on the basis of the calibration curve of gallic acid and expressed as mg/100 mg of the dry weight of SGP-1.

2.9. Antioxidant activity

2.9.1. Hydroxyl radical assay

The hydroxyl radical scavenging ability of SGP-1 was measured according to the method described previously (Halliwell, Gutteridge, & Aruoma, 1987) with a minor modification. Reaction solution mixture in a total volume of 1.0 mL contained phosphate buffer (pH 7.4, 20 mM), EDTA (100 μM), deoxyribose (60 mM), ferric trichloride (100 μM), H_2O_2 (1 mM), ascorbic acid (100 μM), and different concentrations of SGP-1 (0, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL). Solutions of ascorbic acid and ferric trichloride were prepared immediately before use. The reaction solutions were incubated for 30 min at room temperature, and then 1 mL of 1% TBA and 1 mL of 30% (v/v) HCl were added to the mixture. The mixtures were boiled for 10 min and cooled with ice bath. Deionized water and ascorbic acid were added as blank and positive control, respectively. The mixtures were measured at 532 nm, and the scavenging activity of hydroxyl radical (%) was calculated according to the following equation:

$$\text{Scavenging effect } (\%) = \frac{A_{532}(\text{blank}) - A_{532}(\text{sample})}{A_{532}(\text{blank})} \times 100$$

where A_{532} (blank) was the absorbance of the control (deionized water, instead of sample) and A_{532} (sample) was the absorbance of the test sample mixed with reaction solution.

2.9.2. Superoxide radical assay

The superoxide radical scavenging activity of SGP-1 was determined according to the literature procedure (Sun, Wang, Fang, Gao, & Tan, 2004) with a few modifications. Briefly, superoxide radicals were created in 3.0 mL of 16 mM Tris–HCl buffer (pH 8.0), containing 78 mM reduced nicotinamide adenine dinucleotide (NADH), 50 μM nitroblue tetrazolium (NBT), 10 μM phenazin methosulfate (PMS), and samples at given concentrations (0, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL). The colorable reaction of superoxide radicals with NBT was assessed at 560 nm. The deionized water was used as the blank control and ascorbic acid was used as positive control. The scavenging effect of superoxide radicals (%) was calculated according to the following equation:

$$\text{Scavenging effect } (\%) = \frac{A_{560}(\text{blank}) - A_{560}(\text{sample})}{A_{560}(\text{blank})} \times 100$$

where A_{560} (blank) was the absorbance of the control (deionized water, instead of sample) and A_{560} (sample) was the absorbance of the test sample mixed with reaction solution.

2.9.3. Scavenging activity of DPPH radical

The scavenging activity of 1,1-dihpenyl-2-picrylhydrazyl (DPPH) radical was measured according to a literature method (Braca et al., 2001) with slight modification. 0.5 mL of samples (0, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL) was added to 3.0 mL of a 0.01% (v/v) ethanol solution of DPPH. Absorbance at 517 nm was measured after 30 min. The scavenging activity of DPPH radical (%) was calculated according to the following equation:

$$\text{Scavenging effect } (\%) = \frac{A_{517}(\text{blank}) - A_{517}(\text{sample})}{A_{517}(\text{blank})} \times 100$$

where A_{517} (blank) was the absorbance of the control (deionized water, instead of sample) and A_{517} (sample) was the absorbance of the test sample mixed with reaction solution.

2.9.4. Total antioxidant activity

The total radical scavenging capacity was assessed with a reported procedure (Katalinic, Milos, Kulisic, & Jukic, 2006) with slight modification. The ABTS radical cation ($ABTS^+$) was generated by mixing an ABTS (7 mM) solution with a potassium persulfate (2.45 mM) aqueous solution and leaving the mixtures in a dark place at room temperature for 16 h. Then 3.6 mL of the $ABTS^+$ solution was added to 0.4 mL of various concentrations (0, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL) of the SGP-1 solutions. After reacting for 20 min at room temperature, the absorbance was measured at 734 nm. The scavenging activity of ABTS (%) was calculated according to the following equation:

$$\text{Scavenging effect } (\%) = \frac{A_{734}(\text{blank}) - A_{734}(\text{sample})}{A_{734}(\text{blank})} \times 100$$

where A_{734} (blank) was the absorbance of the control (deionized water, instead of sample) and A_{734} (sample) was the absorbance of the test sample mixed with reaction solution.

2.9.5. Determination of Fe^{2+}-chelating ability

The chelating activity of SGP-1 on Fe^{2+} was carried out following a reported procedure (Dinis, Madeira, & Almeida, 1994).

Table 2
Regression coefficients of the predicted quadratic polynomial model.

Sources	Sum of squares	Degree of freedom	Mean square	*F* value	Significance level	
Model	1.72	9	0.19	18.55	0.0004	**[a]
x_1	0.11	1	0.11	10.47	0.0143	*[b]
x_2	0.27	1	0.27	26.53	0.0013	**
x_3	0.42	1	0.42	40.55	0.0004	**
x_1x_2	0.034	1	0.034	3.32	0.1114	
x_1x_3	0.058	1	0.058	5.58	0.0502	
x_2x_3	0.042	1	0.042	4.07	0.0834	
x_1x_1	0.12	1	0.12	11.65	0.0112	*
x_2x_2	0.48	1	0.48	46.19	0.0003	**
x_3x_3	0.012	1	0.12	11.65	0.0112	*
Residue	0.072	7	0.010			
Lack of fit	0.053	3	0.018	3.72	0.1186	
Pure error	0.019	4	4.770E−003			
Cor total	1.80	16				
	R^2 = 0.9597	Adj R^2 = 0.9098	CV = 2.55			

[a] Significance at 0.01 level.
[b] Significance at 0.05 level.

Different concentration of SGP-1 (0, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL) was mixed with 3.7 mL deionized water, and then reacted with $FeCl_2$ (2 mM, 0.1 mL). After 0.2 mL of 5 mM ferrozine was added, the solution was mixed, left to stand for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm. The deionized water and EDTA was respectively measured as the blank and positive control. The chelating activity of SGP-1 on Fe^{2+} (%) was calculated according to the following equation:

$$\text{Chelating ability } (\%) = \frac{A_{562}(\text{blank}) - A_{562}(\text{sample})}{A_{562}(\text{blank})} \times 100$$

where A_{562} (blank) was the absorbance of the control (deionized water, instead of sample) and A_{562} (sample) was the absorbance of the test sample mixed with reaction solution.

2.9.6. Determination of reducing power

The reducing power of SGP-1 was evaluated according to a previous method (Oyaizu, 1986) with slight modification. The reaction mixtures contained 2.5 mL phosphate buffer (pH 6.6, 0.2 M), 2.5 mL potassium ferricyanide (1%, w/v) and SGP-1 (0, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/mL). After incubating at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture for terminating the reaction, and then centrifuged at 1000 × *g* for 10 min. An aliquot of 2.5 mL supernatant was collected and mixed with 2.5 mL deionized water and 0.5 mL $FeCl_3$ (0.1%, w/v). After incubating at room temperature for 15 min, the absorbance of the mixture was measured at 700 nm, using ascorbic acid as a positive control.

2.10. Statistical analysis

All the data were presented as means ± standard deviation (SD) from triplicates. Statistical analysis involved use of the Sigmaplot software package 12.0 (Systat Software Inc.) and Excel 2007 (Microsoft Corp.). Difference was considered significant when $P < 0.05$.

3. Results and discussions

3.1. Optimization of the extraction process

In this study, RSM was introduced to evaluate the multiple parameters and their interactions in a hot water extraction process. Because of saving time, space and raw material, Box Behnken design (BBD) is more advantageous than the traditional single parameter optimization (Box & Behnken, 1960; Qiao et al., 2009). A considerable variation in the yields of SGP, which depended upon the extraction conditions at different experimental combinations were shown in Table 1. By employing multiple regression analysis on the experimental data, the predicted response *Y* for the yield of polysaccharides can be obtained by the following second-order polynomial equation:

$$Y = 4.31 + 0.12x_1 + 0.19x_2 + 0.23x_3 + 0.092x_1x_2 + 0.12x_2x_3 + 0.10x_1x_3 - 0.17x_1^2 - 0.34x_2^2 - 0.17x_3^2 \quad (4)$$

Table 2 listed the fit statistics of polysaccharide extraction yield (*Y*) for the designed polynomial quadratic model. *F*-value of 18.55 and *P*-value of 0.0004 indicates the model was significant. The lack of fit evaluates the model's failure on the points in the designed experimental data domain that were not included in the regression. The low value of 3.72 for *F*-value and 0.1186 for *P*-value of lack of fit elucidated the model was not significant relative to the pure error. The accuracy of the models was evaluated by determination of coefficient R^2 and adjusted coefficient R^2 values. The value of R^2 calculated from ANOVA analysis was 0.9597, indicating that only 4.03% of the total variations were not explained by the model. The adjusted R^2 value of 0.9098 also confirmed that the model was significant. The degree of precision from the compared experiments was exhibited by Coefficient of Variation (CV) value. A low value 2.55 of CV clearly indicated a high degree of precision and a good reliability of the experimental values. The significance of each coefficient and interaction strength between each independent variable was estimated using *P*-values. Data showing in Table 2 indicated that all the linear coefficients (x_1, x_2, x_3) and quadratic term coefficient (x_1^2, x_2^2, x_3^2) significantly affected the yield of SGP, with *P*-values smaller than 0.05. The other term coefficients (x_1x_2, x_2x_3, x_1x_3) were not significant ($P > 0.05$).

3D response surface and 2D contour plots based on the regression equation could provide a process to visualize the relationship between responses and trial levels of each variable and the specific interactions between two test variables. Whether the interactions between the variables are significant or not could be indicated by the shapes of the contour plots. Circular contour plot exhibits that the interactions between the corresponding variables are negligible, while elliptical contour plot shows that the interactions between the corresponding variables are significant (Muralidhar, Chirumamil, Marchant, & Nigam, 2001). As shown in Fig. 1, a high value yield of SGP occur at a high ratio of water to raw material and a moderate temperature (Fig. 1a and d), a high ratio of water to raw material and a long duration (Fig. 1b and e) and a long duration

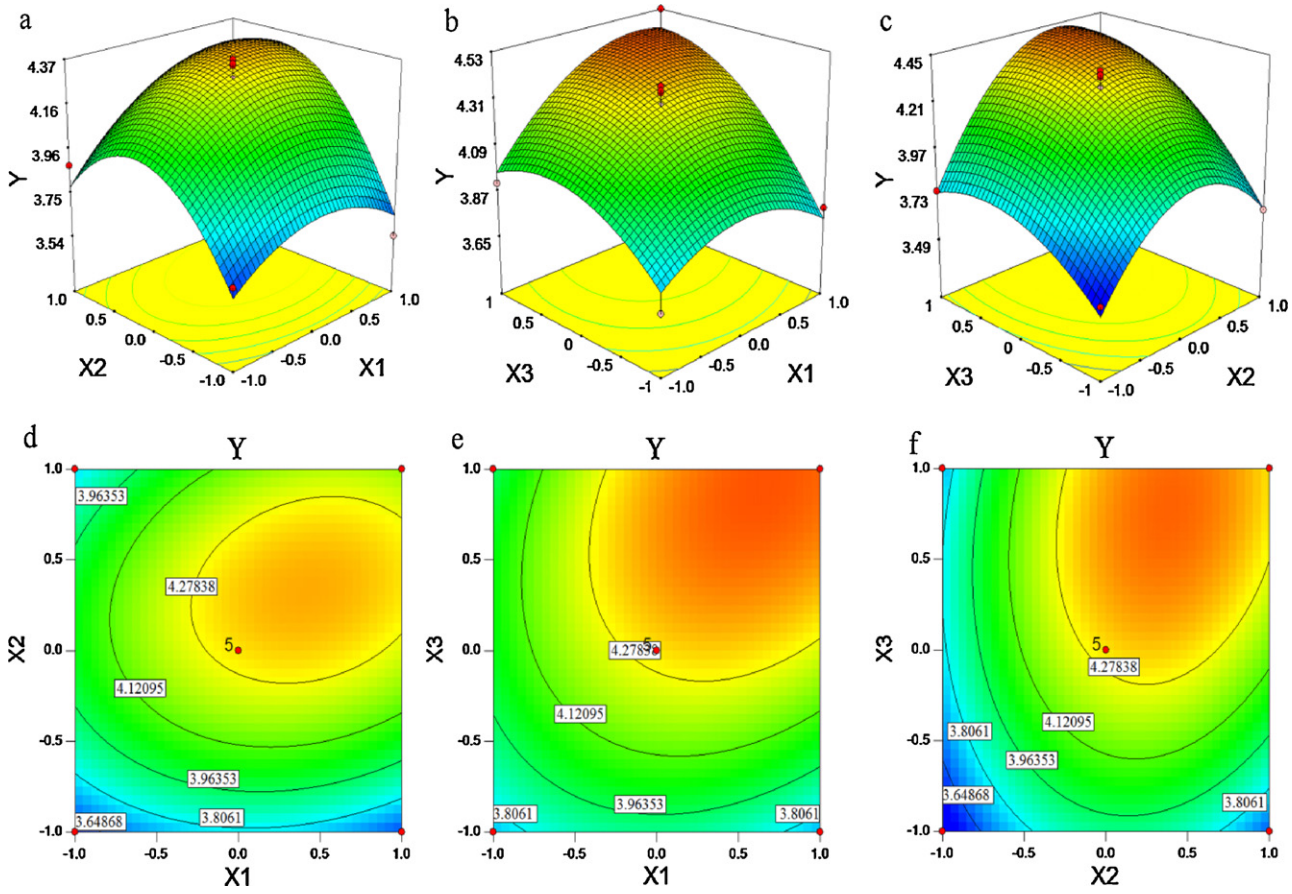

Fig. 1. Response surface (3D) and contour plots (2D) showing the effects of variables (x_1: ratio of water to raw material; x_2: extraction temperature, °C; and x_3: extraction duration, h) on the response *Y*.

and a moderate temperature (Fig. 1c and f). Fig. 1 showed that the response surface of each variable was independent with each other. The interactions of three variables were not significant, which are also observed from the *P*-values in Table 2. The optimal conditions for SGP extraction obtained from these plots were ratio of water to raw material at 30, extracting temperature at 85 °C and extracting duration for 3 h. The maximum predicted yield of SGP under this condition was 4.55%. Three tests were carried out to evaluate the reliability of predicted optimum conditions. Compared with the yield of 4.53 ± 0.07% of the Run 16th in Table 1, the value of 4.49 ± 0.09% was considered not significant difference ($P > 0.05$).

3.2. Homogeneity, molecular weight, carbohydrate content, and monosaccharides composition of SGP-1

The high performance size-exclusive chromatograph (HPSEC) was employed to determine the homogeneity and relative molecular weight of SGP-1, using standard glucans as references. As shown in Fig. 2, SGP-1 was eluted as single and symmetric peak from GPC profile, which indicated that it was homogeneous. The relative molecular weight of SGP-1 was estimated to be 10.6 kDa. Reaction with Coomassie Brilliant G-250 was negative and no absorption at 260 or 280 nm in the UV spectrum indicating the absence of protein and nucleic acid in SGP-1. Total carbohydrate content in SGP-1 was determined to be 95.57%. GC is preferred to monosaccharides quantitative and qualitative determination for its sensitive. Compared to the retention time of the acetate derivatives in GC, SGP-1 consisted of three different monosaccharides, including glucose, galactose, mannose (Fig. 3), and the molar ratio of 8.38:3.13:1.00 was measured by internal standard method.

3.3. FT-IR analysis

The FT-IR spectrum of SGP-1 was shown in Fig. 4, a broad band around 3400 cm^{-1} exhibited O—H stretch vibration and the peak at 2929 cm^{-1} was assigned to C—H stretch vibration. The peak around 1648 cm^{-1} was a characteristic absorption band of the bonded water (Maréchal, 2004). Three stretching peaks at 1026, 1080, and 1155 cm^{-1} indicated the presence of C—O bonds and pyranose ring in the monosaccharide in SGP-1. The moderate intense bands in the region of 1350–1450 cm^{-1} were corresponded to symmetrical deformations of CH_2 and COH groups. The absorption at 892 cm^{-1} belonged to the β-anomeric configuration. The peak at 937 cm^{-1} was belonged to the skeletal mode of pyranose ring (Yi et al., 2012). Those signals all indicated that SGP-1 had the typical saccharide moiety absorption peaks.

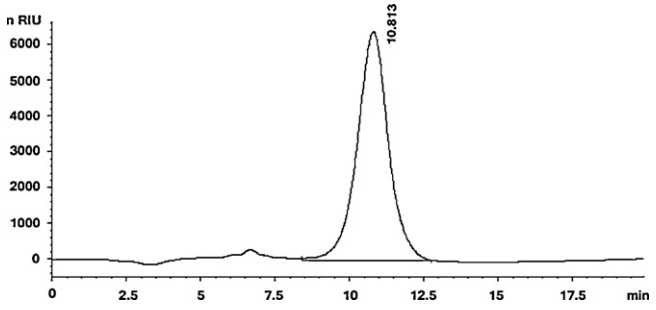

Fig. 2. HPSEC trace of SGP-1 detected by RID.

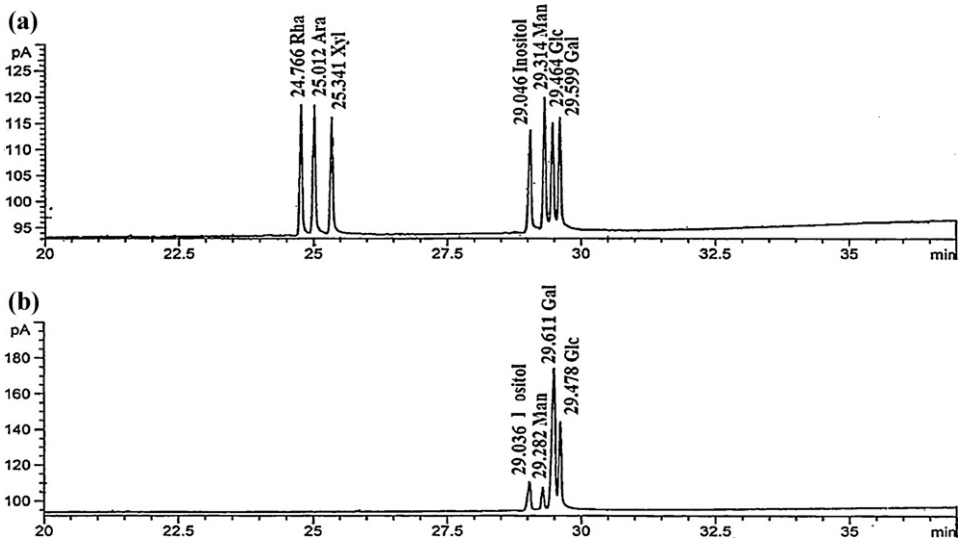

Fig. 3. (a) Relative retention times on GC of acetate derivative of standard monosaccharides. (b) Relative retention times on GC of acetate derivative of monosaccharides in SGP-1.

3.4. Total phenolic content in SGP-1

The compounds extracted from natural resources with good antioxidant activity except of polysaccharides is responsible phenolics as well. The total phenol content of SGP-1 was about 0.124 ± 0.011 mg/100 mg ($n = 5$). For the total phenolics were almost removed by the purification using DEAE-cellulose-52 and Sephacryl S-400 column chromatography, the antioxidant activity of SGP-1 mainly resulted from polysaccharides.

3.5. Antioxidant activity

3.5.1. Scavenging ability of hydroxyl radicals

The hydroxyl radical is generally accepted as a highly potent oxidant. After crossing into cell, hydroxyl radical readily reacts with diverse biomolecules, such as proteins, lipids DNA, and carbohydrates in cells, which accordingly causes tissue damage or cell apoptosis (Yang et al., 2011). Fig. 5a showed the hydroxyl radical scavenging ability of SGP-1 at different concentrations with ascorbic acid as the positive control. SGP-1 exhibited scavenging activity on hydroxyl radicals in a concentration-dependent manner at the test concentrations. At the concentration of 1.0 mg/mL, the highest hydroxyl radical scavenging rate (59.03%) of SGP-1 was achieved, which was 18.23% less compared with that of 1.0 mg/mL ascorbic acid (77.26%). When the concentrations increased from 1.0 mg/mL to 2.0 mg/mL, both ascorbic acid and SGP-1 did not show any higher scavenging effects. The mechanism of SGP-1 on cleaning of the hydroxyl radical could relate to chelate iron and induce them inactive in Fenton reaction.

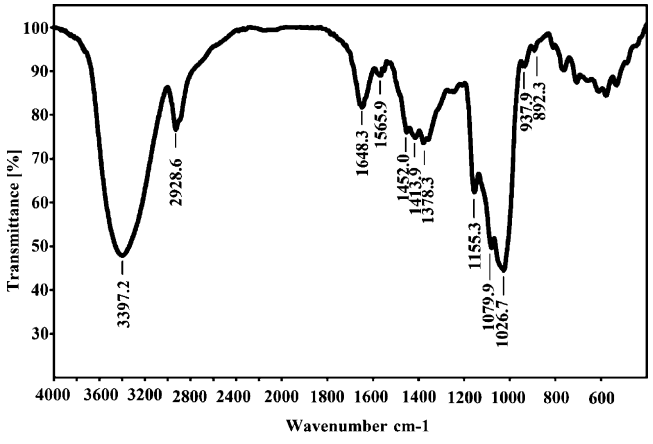

Fig. 4. IR spectra of SGP-1.

3.5.2. Scavenging ability of superoxide anion

Superoxide radical is cell-injuring through causing damage to DNA and membrane lipid of cell (MacDonald, Galley, & Webster, 2003). SGP-1 was compared with ascorbic acid for superoxide radical scavenging activity. As shown in Fig. 5b, at the test concentrations, SGP-1 exhibited scavenging activity on superoxide radicals in a concentration-dependent manner. The maximum scavenging ability (70.82%) was observed for SGP-1 at a concentration of 1.0 mg/mL, while ascorbic acid could achieve the maximum scavenging activity of 90.46% at 0.25 mg/mL. At low concentrations (0–0.5 mg/mL), ascorbic acid exhibit much higher scavenging activity than SGP-1. However, as concentration increased to up to 1.0 mg/mL, SGP-1 showed strong scavenging ability. Polysaccharides with special conformations, hydrogen in O—H bonds could be easily liberated and thus could stabilize superoxide anion. The mechanism of SGP-1 on scavenging superoxide anion may be associated with the dissociation energy of O—H bond.

3.5.3. DPPH radical scavenging activity

The antioxidant mechanism of DPPH radical scavenging is related to the acceptance of hydrogen by the DPPH radical. With the hydrogen donated by the antioxidant, the DPPH was converted into DPPH-H, a non-radical form. The model of scavenging DPPH radical is well acknowledged and widely applied to estimate the free radical scavenging ability of various antioxidants (Yuan, Zhang, Fan, & Yang, 2008). The hydrogen-donating ability of these antioxidants decides their antioxidant capacity. Fig. 5c depicted DPPH radical scavenging activity of SGP-1 and compared with ascorbic acid as positive control. Under the experimental conditions, the scavenging effect of SGP-1 was correlated well with the increasing concentrations. The DPPH radical scavenging rate of SGP-1 increased from 10.87% to 77.59%, at the concentrations from 0.0625 to 2.0 mg/mL. The result indicated that SGP-1 had noticeable effect on the scavenging of free DPPH radicals. However, the scavenge effect of ascorbic acid is stronger than that of SGP-1 on the identical concentration.

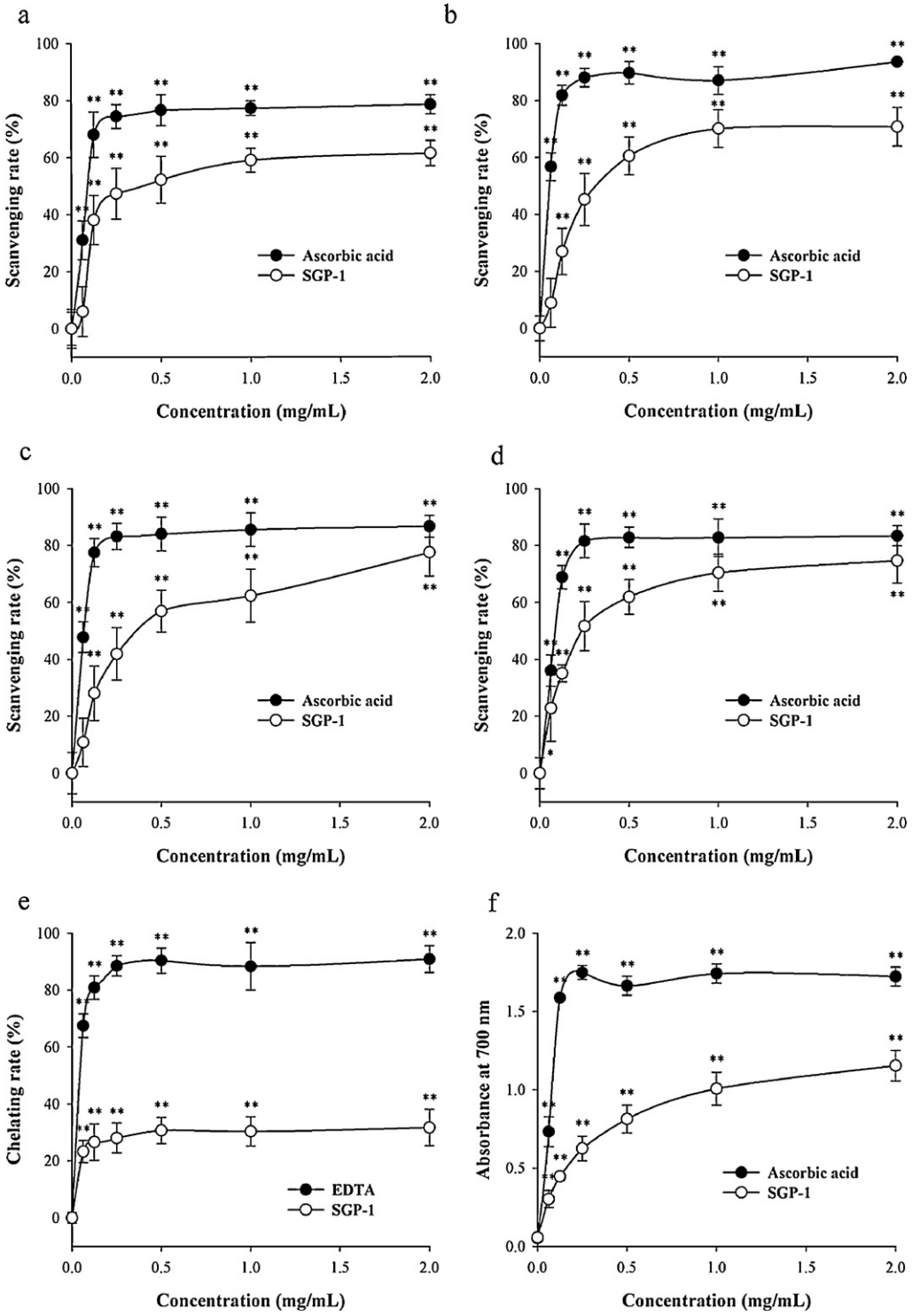

Fig. 5. Antioxidant activities of SGP-1. (a) Scavenging ability of hydroxyl radicals, (b) scavenging ability of superoxide anion, (c) DPPH radical scavenging activity, (d) ABTS radical scavenging activity, (e) ferrous ion chelating ability, and (f) reducing power. All treatments were conducted in triplicate, and mean values are reported. The vertical bars represent the standard deviation of each data point. Values marked by the same code are significantly different compared with the blanks ($P < 0.01$).

3.5.4. ABTS radical scavenging activity

ABTS assay is an accepted method in measuring the total antioxidant power of a potential antioxidant. The scavenging ability of SGP-1 and ascorbic acid on ABTS free radicals is shown in Fig. 5d. The scavenging power of SGP-1 correlated well with the increasing concentrations, but was slightly lower than ascorbic acid at the same concentration. The maximum scavenging ability of ascorbic acid was at the concentration of 0.5 mg/mL, while SGP-1 obtained the maximum effect at the concentration of 2.0 mg/mL. SGP-1 exhibited more than 50% scavenging ABTS radical capacity since the concentration reached 0.5 mg/mL. The results indicated that SGP-1 had strong scavenging power for ABTS radical and could be explored as a potential antioxidant. This assay can be used in both organic and aqueous solvent systems and can also be used as an index to reflect the antioxidant activity of the test samples (Aparicio, Peinado, Escrig, & Rupérez, 2010; Luo et al., 2010).

3.5.5. Ferrous ion chelating ability

The metal chelating ability was recognized as a correlative activity to antioxidant. Sometimes samples' antioxidant capacity could also be increased by enhancing their metal chelating activity. The ferrous ion chelating activity of SGP-1 at different concentrations is shown in Fig. 5e. Compared with EDTA in the equivalent concentration, SGP-1 exhibited a much weaker metal chelating ability. The chelating rate for SGP-1 was only 31.73% even at 2.0 mg/mL, while that of EDTA was 90.90%. Several studies have demonstrated that the metal ion chelating ability of polysaccharides could be due to formation of cross-bridge between carboxyl group in uronic acid and divalent ion, and difference in ferrous ion chelating ability among various acidic polysaccharides was minor. However, the result of monosaccharide composition did not show SGP-1contain any carboxyl group (Fan, Li, Deng, & Ai, 2012). The role of SGP-1 in the process of ferrous ion chelating needs to be further explored.

3.5.6. Reducing power

The reducing power, which serves as a significant potential activity index, could be assessed by a Fe^{3+}–Fe^{2+} reduction reaction. The presence of reductant in the reaction causes the reduction of Fe^{3+}/ferricyanide complex to the ferrous form and can be monitored by the formation of Perl's Prussian blue at 700 nm. The reducing power of SGP-1 and ascorbic acid was compared in Fig. 5f, higher absorbance value indicates stronger reducing power. The maximum reducing power of ascorbic acid and SGP-1 were obtained at 0.25 mg/mL (absorbance = 1.749) and 2.0 mg/mL (absorbance = 1.178), respectively. There are various mechanisms to explain the activities of antioxidants, such as decomposition of peroxide, prevention of continued hydrogen abstraction, prevention of chain initiation, and chelating of transition metal ions. Sun, Liu, and Kennedy (2010) reported that antioxidant polysaccharide could be defined as electron donors, which could react with free radicals. The moderate reducing power of SGP-1 showed that SGP-1 may has some free electrons to react with Fe^{3+}. However, the reducing power of SGP-1 was much weaker compared with that of ascorbic acid.

4. Conclusion

Response surface methodology was an effective tool for optimizing conditions of SGP extracted from *S. glabra*. The optimal experimental extraction yield $4.49 \pm 0.09\%$ was obtained when the extraction parameters as following conditions, ratio of water to raw material at 30, extraction temperature at 85, and the extraction duration at 3 h. Under the optimum condition, the experimental extraction yield of SGP agreed closely with the predicted yield of 4.55%. After purified by DEAE-cellulose-52 and Sephacryl S-400 gel chromatography, a homogenous polysaccharide SGP-1 was obtained. The average molecular weight of SGP-1 was 10.6 kDa, which was detected by the HPSEC. The monosaccharide composition study demonstrated that SGP-1 is a heteropolysaccharide consisting of glucose, galactose, and mannose in the ratio of 8.38:3.13:1. The result of *in vitro* antioxidant measurement demonstrated that SGP-1 can scavenge free radicals, which may contribute to the ability of *S. glabra* to treat the inflammation disease caused by oxidant damage.

However, further investigation of structural identifications is required to elucidate the antioxidant mechanism.

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