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Optimization of extraction, preliminary characterization and hepatoprotective effects of polysaccharides from *Stachys floridana* Schuttl. ex Benth

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ABSTRACT

Optimization of extraction, preliminary characterization and hepatoprotective activity of polysaccharides from *Stachys flordana* Schuttl. ex Benth. (SFPS) were investigated in the present study. Firstly, the optimal conditions for extraction of SFPS were obtained by a Box-Behnken design as follows: extraction temperature 94 °C, extracting time 4 h and ratio of water to material 19 mg/ml. Then, the analysis of monosaccharide composition by gas chromatography revealed that SFPS was composed of rhamnose, arabinose, glucose and galactose in a molar percent of 2.05, 9.16, 28.66 and 60.14, respectively. Finally, we demonstrated that SFPS had a significant protective effect against acute hepatotoxicity induced by carbon tetrachloride (CCl_4) in mice, as evident by lower levels of serum alanine aminotransferase, aspartate aminotransferase and hepatic malondialdehyde, higher activities of superoxide dismutase and catalase, as well as higher reduced glutathione concentration compared to the CCl_4-treated group. The results suggested that SFPS could be explored as novel natural supplement.

1. Introduction

Stachys L., one of the largest genera of the Lamiaceae family, contains more than 270 species (Ball, 1972). Many species of this genus have been used medicinally for centuries to treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough, ulcers and infected wounds (Conforti et al., 2009; Hartwell, 1982; Skaltsa, Lazari, Chinou, & Loukis, 1999). Furthermore, recent investigations on different taxa of this genus have demonstrated that extracts of *Stachys* species exert various pharmacological effects, such as anti-inflammatory, antitoxic, antibacterial, antioxidant and cytotoxic activities (Grujic-Jovanovic, Skaltsa, Marin, & Sokovic, 2004; Haznagy-Radnai et al., 2008; Khanavi, Sharifzadeh, Hadjiakhoondi, & Shafiee, 2005; Salehi, Sonboli, & Asghari, 2007; Vundac, Brantner, & Plazibat, 2007). *Stachys floridana* Schuttl. ex Benth., distributed mainly in Henan Province of China, is a perennial herbaceous plant that belongs to the *Stachys* genus. Its rhizome, known as "Yintiao" in China, is a delicious vegetable with local characteristics and often processed into many kinds of foods by frying, saucing or preserving (Zhang, Li, & Cheng, 2003). In recent years, it has been used in the preparation of foods such as yoghurt and jelly to improve the taste (Bendahou, Dufresne, Kaddami, & Habibi, 2007; Yi et al., 2007). In addition, it is rich in carbohydrate, polyphenols, vitamin C, protein and organic acid that may contribute to the biological functions, such as anti-hyperlipidemic effect, softening blood vessels and improving blood circulation (Li, Liang, & Dong, 2010). However, little attention has been devoted to the extraction and biological functions of polysaccharides from *S. floridana Schuttl.* ex Benth. (SFPS). Therefore, we report here the optimization of extraction, preliminary characterization and hepatoprotective effects of SFPS on carbon tetrachloride (CCl_4)-induced acute liver damage in mice.

In recent years, response surface methodology (RSM) has been extensively used to optimize medium composition of fermentation process, conditions of enzyme reaction, extraction conditions for bioactive compounds and food processing methods (Kshirsagar & Singhal, 2007; Masmoudi et al., 2008; Qiao et al., 2009; Sun, Liu, & Kennedy, 2010a). The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions (Chen, Chen, & Lin, 2005; Ma, Wang, & Wu, 2010). Therefore, Box-Behnken design (BBD), one of RSM, was used in the present study to determine the optimum conditions for

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Table 1
The Box-Behnken design matrix and response values for extraction yield of SFPS.

Run number	Extraction temperature (X_1: °C)	Ratio of water to material (X_2: ml/g)	Extraction time (X_3: h)	Polysaccharide yield (%)	
				Experimental	Predicted
1	−1 (85)	0 (15)	−1 (3)	4.70 ± 0.54	4.82
2	1 (95)	0 (15)	−1 (3)	7.52 ± 0.51	7.36
3	−1 (85)	0 (15)	1 (5)	6.87 ± 0.36	7.03
4	1 (95)	0 (15)	1 (5)	10.07 ± 0.27	9.95
5	−1 (85)	−1 (10)	0 (4)	5.71 ± 0.63	5.57
6	1 (95)	−1 (10)	0 (4)	7.68 ± 0.41	7.81
7	−1 (85)	1 (20)	0 (4)	6.10 ± 0.52	5.97
8	1 (95)	1 (20)	0 (4)	9.05 ± 0.40	9.19
9	0 (90)	0 (15)	−1 (3)	4.54 ± 0.16	4.57
10	0 (90)	0 (15)	1 (5)	8.74 ± 0.30	8.72
11	0 (90)	1 (15)	−1 (3)	7.20 ± 0.13	7.22
12	0 (90)	1 (15)	1 (5)	7.90 ± 0.24	7.87
13	0 (90)	0 (15)	0 (4)	8.80 ± 0.30	8.98
14	0 (90)	0 (15)	0 (4)	8.79 ± 0.37	8.98
15	0 (90)	0 (15)	0 (4)	9.05 ± 0.21	8.98
16	0 (90)	0 (15)	0 (4)	9.09 ± 0.17	8.98
17	0 (90)	0 (15)	0 (4)	9.18 ± 0.19	8.98

extraction of SFPS in order to obtain a high extraction yield. Then, SFPS was preliminary characterized by gas chromatography (GC) and Fourier transform-infrared spectroscopy (FT-IR). Finally, the hepatoprotective effects of crude SFPS against CCl_4-induced acute liver damage in Kuming mice were investigated.

2. Materials and methods

2.1. Materials

The fresh rhizomes of *S. floridana* Schuttl. ex Benth., purchased from a local market in Henan Province of China, were washed with distilled water, dried and ground into powder using a high speed disintegrator, and the material that passed through a 60-mesh sieve was then kept in sealed polyethylene bags until use. The male Kunming mice were obtained from the Experimental Animal Centre of Academy of the Military Medical Sciences (Beijing, China). Arabinose, rhamnose, fucose, xylose, galactose, glucose and mannose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Analytical kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA) and total antioxidant capacity (TAC) were obtained from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). All other chemicals and reagents were analytical grade.

2.2. Preparation of crude SFPS

The extraction of SFPS was performed according to the reported method with some modifications (Zhao, Dong, Chen, & Hu, 2010). Briefly, the powder of dried rhizomes was refluxed with 85% ethanol at 90 °C for 2 h. The extract was removed, and the resulting pretreated powder was dried and used for the extraction of SFPS. The pretreated sample was extracted by distilled water in a designed manner as follows: ratio of water to raw material (v/w), 5–25; extraction temperature, 75–100 °C and extraction time, 1–5 h. The extraction mixture was centrifuged at 5000 rpm for 15 min, and the insoluble residue was retreated as mentioned above for 2–4 times. The supernatants were collected, concentrated by a rotary evaporator under reduced pressure to an appropriate volume, mixed with three times of absolute ethanol and kept overnight at 4 °C. The resulting precipitates were collected by centrifugation at 5000 rpm for 20 min, washed sequentially with anhydrous ethanol and acetone, and dried to afford crude SFPS. The extraction yield was calculated according to the following formula:

$$\text{Extraction yield (\%)} = \frac{W_1}{W_0} \times 100$$

where W_1 and W_0 are the weights of crude SFPS and pretreated sample respectively.

2.3. Box-Behnken design for SFPS extraction

On the basis of single-factor experiment for the polysaccharide extraction, proper ranges of extraction temperature, extraction time and ratio of distilled water to material were determined. Then, a 17-run BBD with three factors and three levels including five replicates at the centre point was used to optimize the extraction conditions. As shown in Table 1, the three factors (extraction temperature, extraction time and ratio of distilled water to material) were designated as X_1, X_2 and X_3, and three levels were coded as +1, 0, and −1 for high, intermediate and low values, respectively. Each extraction was run in triplicate, and the extraction yield was expressed as mean ± standard deviation (SD).

For statistical calculation, the variables were coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X_i}$$

where x_i is the coded value of an independent variable, X_i is the corresponding actual value of an independent variable, X_0 is the actual value of an independent variable at centre point, and ΔX_i is step change value of an independent variable. A second-order polynomial mode was fitted to analyze the relationship between the independent variables and the response for extraction yield of SFPS in BBD (Table 1):

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

where Y is the dependent variable (extraction yield of SFPS), X_i and X_j are the coded independent variables ($i \neq j$), β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively.

2.4. Determinations of the contents of carbohydrate, protein, uronic acid and sulfate

The neutral carbohydrate content in SFPS was determined by phenol-sulfuric acid method using D-glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The protein content was determined by the method described by Bradford (1976), and bovine serum albumin was used as the standard. The content of uronic acid was determined according to a *m*-hydroxydiphenyl colorimetric method by using D-glucuronic acid as the standard (Blumenkrantz & Asboe-Hansen, 1973). The content of sulfate radical was determined according to the reported method (Doigson & Price, 1962).

2.5. Analysis of monosaccharide composition of SFPS

The monosaccharide composition of SFPS was analyzed according to the reported method (Jiang, Wang, Liu, Gan, & Zeng, 2011). Briefly, crude SFPS (5.0 mg) was hydrolyzed with 2.0 ml 2 M trifluoroacetic acid at 120 °C for 2 h. The resulting hydrolyzate was repeatedly co-concentrated with methanol to dryness and converted into aldonitrile acetates by the addition of a mixture of methanol, pyridine and acetic anhydride. In a similar manner, the standards of monosaccharide were acetylated. Then, all the derivatives were analyzed by a 6890N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with flame-ionization detector (FID) and an HP-5 fused silica capillary column (30 m × 0.32 mm × 0.25 mm). The operation conditions of GC were as follows: flow rates of N_2, H_2 and air were 25, 30 and 400 ml/min, respectively; the temperatures of oven, detector and inlet were set at 210, 280, 250 °C respectively.

2.6. FT-IR spectrometric analysis

The FT-IR spectrum was recorded on a Bomen MB154S FT-IR spectrometer (ABB Bomen, Inc., Quebec, Canada). The dried SFPS was grinded with potassium bromide powder and pressed into pellet for spectrometric measurement in the frequency range of 4000–500 cm^{-1}.

2.7. Evaluation of hepatoprotective effect of SFPS

2.7.1. Animal grouping and experimental design

Hepatoprotective effect of SFPS against CCl_4-induced acute liver damage in mice was evaluated according to the reported method (Wang, Lou, Wu, & Guo, 2010) with proper modifications. Furthermore, all procedures involving animals were conducted in strict accordance with the Chinese legislation on the use and care of laboratory animals during the entire experimental period (including acclimation). Briefly, male Kunming mice (2-month-old), grade of specific pathogen free with body weight (BW) of 20 ± 2 g, were used in the present study. They were maintained under controlled temperature (23 ± 0.5 °C), humidity (55 ± 5%) and 12-h light/dark cycle and free access to both food (standard rat chow) and water. After adapting to their environment for 1 week, these mice were randomly divided into six groups of 8 each: (a) normal control group; (b) CCl_4 model control group; (c) positive control group, (d–f) SFPS treatment groups. Mice in normal control group and CCl_4 model control group were given physiological saline (10 ml/kg BW, p.o.) once daily. Mice in positive control group were given silymarin (10 mg/kg BW, p.o.) once daily. Mice in SFPS treatment groups were respectively fed with SFPS solution (dissolved in physiological saline) in three different doses (100, 200 and 400 mg/kg BW, p.o.) per day. All administrations were conducted for 10 consecutive days. Three hours after the last administration, all mice except those in normal control group were received an intraperitoneal injection of CCl_4 (10 ml/kg BW of 0.2% CCl_4 solution in olive oil), while the mice in normal control group were treated an equal amount of olive oil alone by intraperitoneal injection. Then all the animals were fasted for 16 h and were subsequently used for the following biochemical analysis.

2.7.2. Biochemical assay

After 16 h fast, the animals were anesthetized with aether and blood samples were taken from their eyepit. The blood samples were kept at room temperature for 1 h and then centrifuged at 4000 × *g* for 10 min to afford serums. Livers were excised, weighed and homogenized immediately in ice-cold 0.9% NaCl solution (0.1 g tissue/ml solution). The suspensions were centrifuged, and the resulting supernatants were collected for further analysis. All above treatments were done at 4 °C.

The activities of AST and ALT in serums and the activities of CAT and SOD, protein contents, levels of GSH, MDA and TAC in livers were determined by using commercially available analytical kits according to the kits instructions.

2.8. Statistical analysis

All data presented are means ± SD of three determinations. Stat-Ease Design-Expert 7.0.0 software (Trial Version, Stat-Ease Inc., MN, USA) was used for the experimental design, data analysis and model building. SPSS 13.0 software Inc. (Chicago, IL, USA) was used for statistical calculations and correlation analysis. Differences were considered to be statistically significant if $P < 0.05$.

3. Results and discussion

3.1. Effect of extraction temperature on the yield of SFPS

The effect of extraction temperature on the yield of SFPS is shown in Fig. 1A. The extraction yield of SFPS increased along with the increase of extraction temperature from 75 °C to 100 °C, which was in accordance with other reports for polysaccharides extraction (Vinogradov, Brade, Brade, & Holst, 2003; Ye & Jiang, 2011). However, we noted that the extraction yield of SFPS increased slowly when the extraction temperature exceeded 90 °C. Therefore 90 °C was selected as the centre point for BBD experiment.

3.2. Effect of ratio of water to material on the yield of SFPS

Fig. 1B shows the effects of ratio of water to material (5, 10, 15, 20, and 25 ml/g) on the extraction yield of SFPS. The yield of SFPS increased from 1.20 to 5.25% as the ratio of water to material increased from 5 to 25 ml/g. This might be due to the increase of the driving force for the mass transfer of the polysaccharides with higher ratio of water to material (Bendahou, Dufresne, Kaddami, & Habibi, 2007). Taking it into consideration that there were no significant differences among 15, 20 and 25 ($P > 0.05$), 15 was selected as the centre point for further experiment.

3.3. Effect of extraction time on the yield of SFPS

Extraction time is an important factor that affects the efficiency of polysaccharide extraction. Therefore, the effects of different extraction times on SFPS extraction were investigated. As shown in Fig. 1C, the yield of SFPS reached a maximum value of 4.80% at an extraction time of 4 h. However, the yield of SFPS decreased slightly over 4 h of extraction. This might be due to the change of polysaccharides molecule structure induced by excessive lengthening of extraction time (Cai, Gu, & Tang, 2008). Thus, the centre point of extraction time chosen for BBD was 4 h.

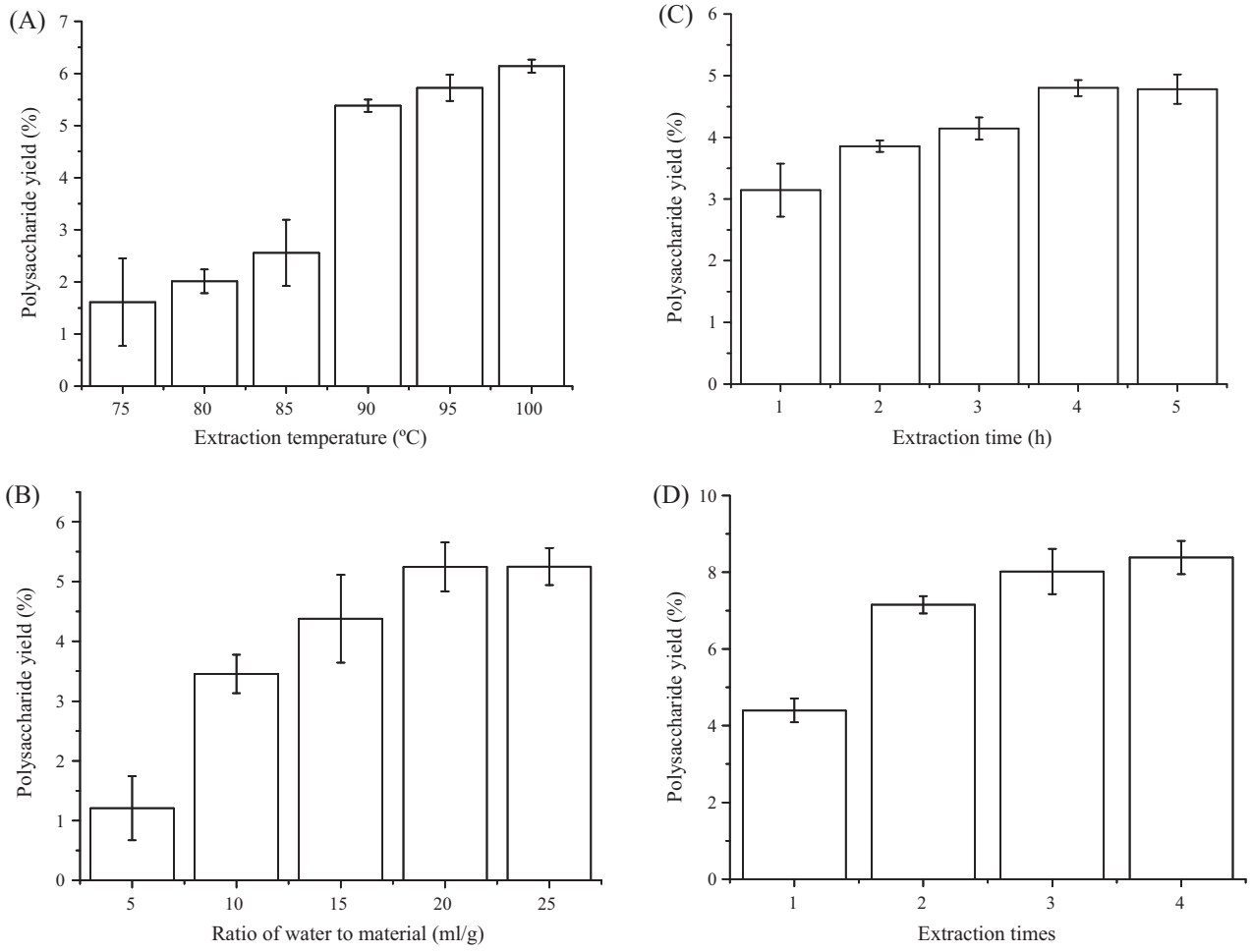

Fig. 1. Effects of different extraction parameters (extraction time (h); extraction temperature (°C); ratio of water to material (ml/g); extraction times) on yield of SFPS.

3.4. Effect of extraction times on the yield of SFPS

Fig. 1D shows the effect of extraction times on yield of SFPS. We found that the yield of SFPS increased greatly accompanying the increase of extracting times, and the yield of SFPS reached a maximum value when the sample was extracted for 4 times. However, there was no significant difference between 3 and 4 times ($P > 0.05$). For saving of energy and lowering of cost, 3 times of extraction was set in the following BBD experiments.

3.5. Optimization by RSM

3.5.1. Model fitting

Based on the results of single-factor experiments, a 17-run BBD with three factors and three levels including five replicates at the centre point were utilized to determine the optimal levels for extraction temperature, ratio of water to material and extraction time. The extraction yields of SFPS under different conditions are shown in Table 1, and the data were analyzed by Design Expert software. As a result, the second-order polynomial equation that revealed the relationship between the yield of SFPS and the test variables was obtained as follows:

$$Y = 5.95441X_1 + 1.63382X_2 + 6.83087X_3 + 3.80546E - 003X_1X_2 + 0.048901X_1X_3 - 0.17485X_2X_3 - 0.032967X_1^2 - 0.034552X_2^2 - 1.02017X_3^2 - 299.87196$$

where Y represents the extraction yield of SFPS, X_1, X_2 and X_3 represent the extraction temperature, ratio of water to material and extraction time, respectively.

In order to ensure a good model, test for significance of the regression model, test for significance on individual model coefficients and test for lack-of fit need to be performed. As shown in Table 2, the high F-value (116.05) and low P-value ($p < 0.0001$, indicating that there was only a 0.01% chance that the model F-value

Table 2
Analysis of variance for response surface quadratic model obtained from experimental results.

Source	Sum of squares	DF	Mean square	F-value	P-value
Model	34.28	9	3.81	116.05	<0.0001[a]
X_1	14.92	1	14.92	362.36	<0.0001[a]
X_2	11.56	1	11.56	280.67	<0.0001[a]
X_3	1.6	1	1.6	38.97	<0.0001[a]
X_1^2	2.86	1	2.86	69.47	<0.0001[a]
X_2^2	3.14	1	3.14	76.31	<0.0001[a]
X_3^2	4.38	1	4.38	106.45	<0.0001[a]
X_1X_2	0.036	1	0.036	0.88	0.3796[b]
X_1X_3	0.24	1	0.24	5.81	0.0468[a]
X_2X_3	3.06	1	3.06	74.27	<0.0001[a]
Lack of fit	0.13	3	0.043	1.68	0.3074[b]
Residual	0.23	7			
Pure error	0.10	4			
Cor total	34.51	16			

$R^2 = 0.9933$, Adjusted $R^2 = 0.9848$.
[a] 5% significance level.
[b] Not significant relative to the pure error.

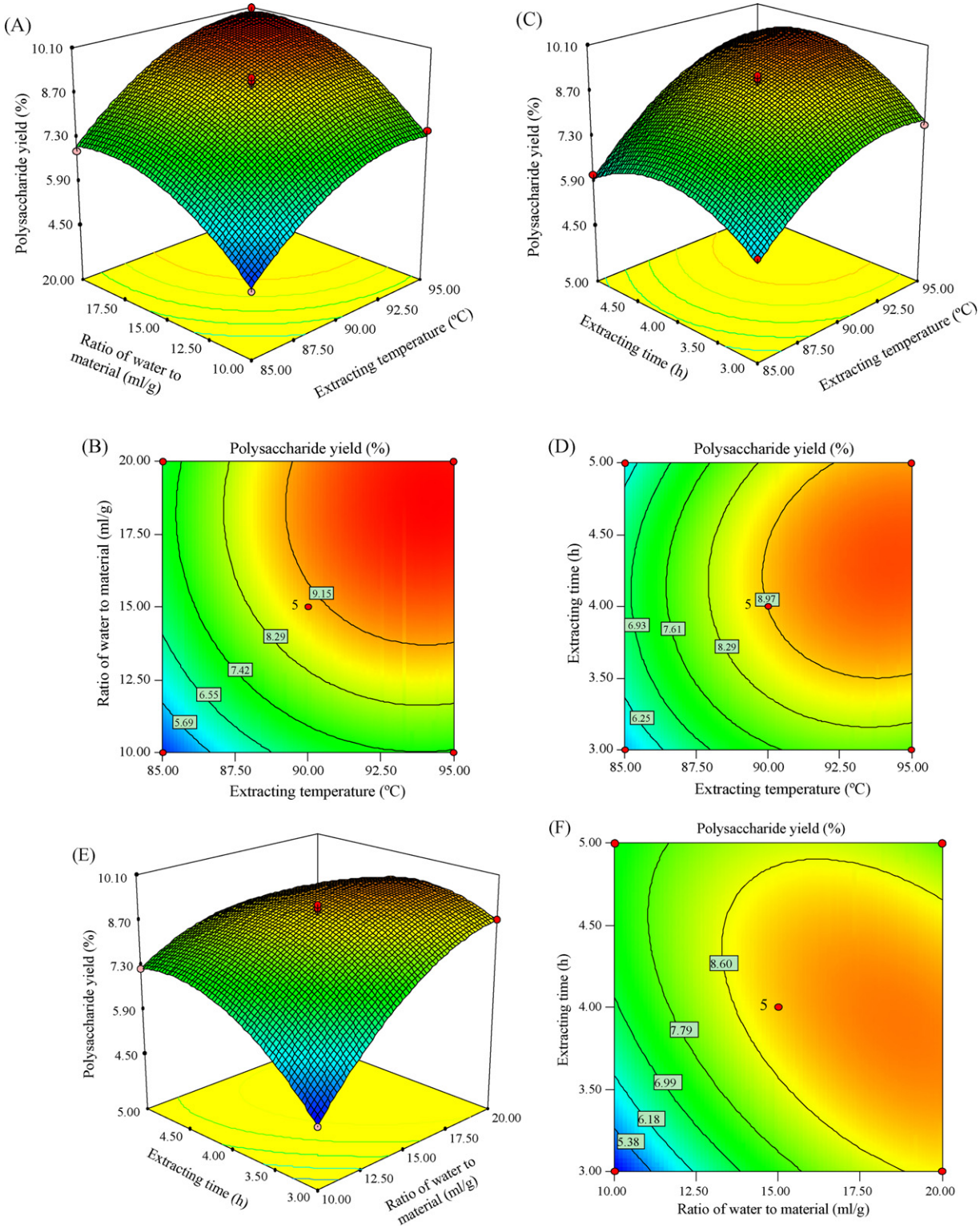

Fig. 2. Response surface plots (A, C and E) and contour plots (B, D and F) showing the effects of extraction temperature, extraction time, ratio of water to material and their mutual effects on extraction yield of SFPS.

could occur due to noise) indicated that the model was highly significant. The value of 1.68 for lack-of-fit implied that the lack-of-fit was insignificant relative to the pure error due to noise, indicating the model equation was adequate to predict the extraction yield of SFPS within the range of experimental variables. In addition, the fitness of the model was further confirmed by the determination coefficient (R^2). In the present study, the value of R^2 (0.9848) was close to 1, which implied that 98.48% of the variability in the data could be explained by the model.

The significance of the regression coefficients can be seen from the *P* values listed in Table 2. Smaller the *P*-value is, more significant the corresponding coefficient is (Jiang et al., 2011). Accordingly, the linear model terms (X_1, X_2 and X_3), quadratic model terms (X_1^2, X_2^2 and X_3^2) and interactive model terms (X_1X_3 and X_2X_3) significantly

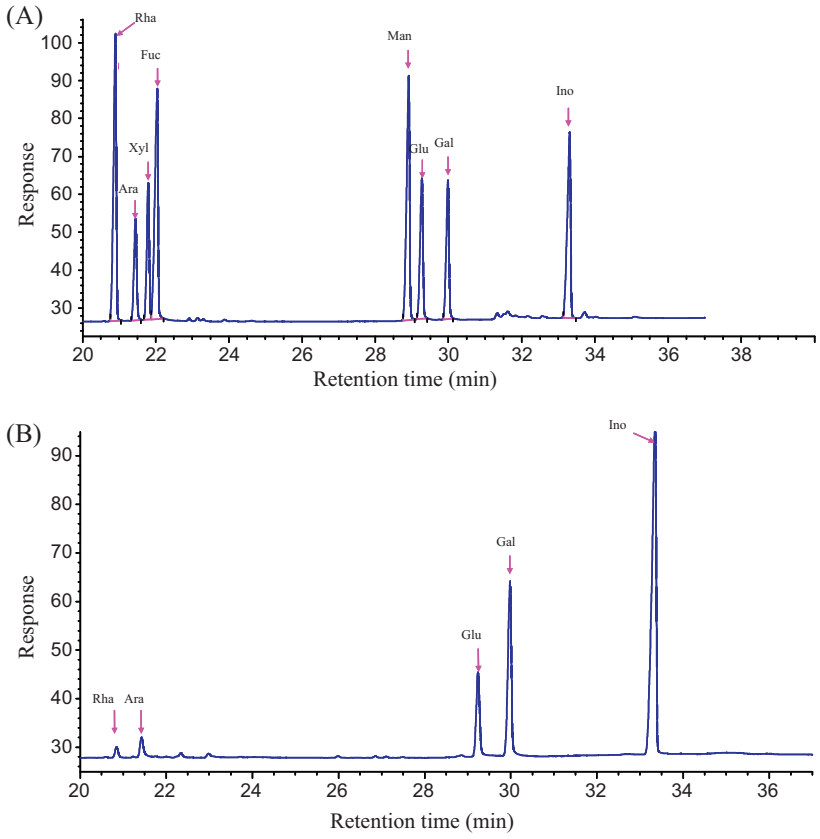

Fig. 3. GC chromatograms of standard monosaccharides (A) crude SFPS (B).

affected the extraction yield of SFPS ($P < 0.05$). Meanwhile, significant interactions between extraction temperature and extraction time and between ratio of water to material and extraction time were observed. The results also indicated that extraction temperature was the most significant single parameter influencing the extraction yield of SFPS followed by ratio of water to raw material and extraction time.

3.5.2. Optimization for SFPS extraction

The 3D response surface and 2D contour plots simulated by Design-Expert software are the graphical representations of regression equation, and the interactions of the variables and as well as the optimal level of each variable for the maximum response can be determined according to the response surface curves. The shape of the contour plot indicates whether the interaction between variables is significant or not. A circular contour plot indicates that the interaction between related variables is negligible, while an elliptical contour plot indicates that the interaction between related variables is significant (Muralidhar, Chirumamila, Marchant, & Nigam, 2001). Fig. 2 plots out graphically the regression equation, which depicted the interactions between two variables by fixing the third variable at its zero level for extraction yield of SFPS. Among the tested three variables, the interactions between extraction temperature and extraction time and between ratio of water to material and extraction time were significant. However, the interaction between ratio of water to material and extraction temperature was not significant, which was in agreement with the data in Table 2.

One objective of the optimization was to determine the optimal conditions that gave the maximum extraction yield of SFPS. Therefore, the optimum levels of the variables were obtained by analyzing the response surface contour plots using Design Expert software. The optimal extraction conditions that provided maximum extraction yield of SFPS were as follows: 94.36 °C, 18.71 ml/g and 4.01 h for extraction temperature, ratio of water to material and extraction time, respectively. Under the optimum conditions, the predicted value for SFPS yield was 10.02%. In order to facilitate the practical extraction process of SFPS, the optimal conditions were modified as follows: extraction temperature of 94 °C, ratio of water to material of 19 ml/g and extraction time of 4 h. In order to validate the adequacy of the model equation, additional verification experiments were performed. As a result, an actual value of $9.98 \pm 0.60\%$ ($n = 5$) was obtained under the optimal extraction conditions, which was in good agreement with the predict value. The result suggests that the regression model is accurate and adequate for the extraction of SFPS.

3.6. Preliminary characterization of SFPS

In the present study, crude SFPS was prepared through a series procedure of hot water extraction based on the optimal extraction conditions, centrifugation, ethanol precipitation and drying. Then, the crude SFPS was preliminary characterized by chemical analysis, GC and FT-IR.

The contents of neutral sugars, protein, sulfate radical and uronic acid in SFPS were determined to be 68.86, 0.74, 1.22 and 27.08%, respectively. Notably, it contained high content of uronic acid. The monosaccharide composition of SFPS was revealed by GC analysis (Fig. 3). We found that SFPS was consisted of rhamnose, arabinose, glucose and galactose in a molar percent of 2.05, 9.16, 28.66 and 60.14, respectively. The results indicated that galactose and glucose were the main monosaccharide compositions of SFPS.

The FT-IR spectrum of SFPS is shown in Fig. 4. The strong bands at 3369.94 and 2928.89 cm^{-1}, two characteristic absorptions of

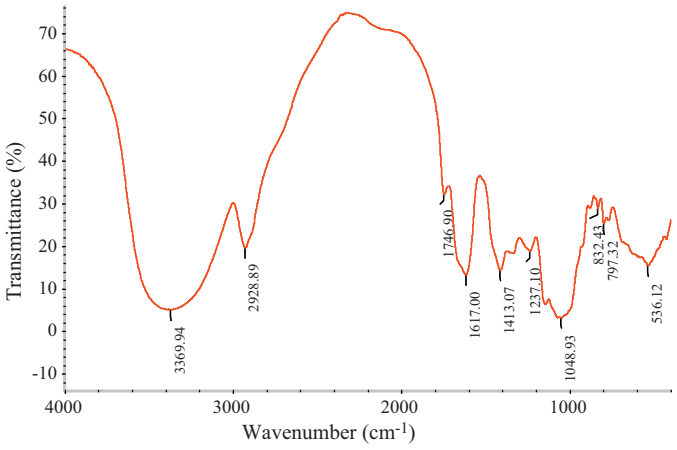

Fig. 4. FT-IR spectrum of crude SFPS.

polysaccharides that attributed to the hydroxyl stretching vibration and C–H stretching vibration of the polysaccharide respectively (Qiao et al., 2009), were observed. In addition, an asymmetrical stretching peak at 1617.00 cm^{-1} and a weak symmetrical stretching peak near 1413.07 cm^{-1} indicated that there were carboxyl groups in SFPS (Mao, Li, Gu, Fang, & Xing, 2004). The result was in good agreement with that of chemical analysis, since SFPS had high content of uronic acid (27.08%) as mentioned above. The absorption at 1237.10 cm^{-1} was related to S=O stretching vibration of the sulfate group (Percival & Wold, 1963). Furthermore, an additional sulfate absorption band at 832.43 cm^{-1} (C–O–S) indicated that the sulfate groups were axial (Bilan, Grachev, Shashkov, Nifantiev, & Usov, 2006) and most sulfate groups were located at positions 2 and 3. The bands between 1143.96 and 1048.93 cm^{-1} in the spectrum were assigned to the valent vibrations of the C–O–C bond and glycosidic bridge (Zhao, Yang, Yang, Jiang, & Zhang, 2007).

3.7. Hepatoprotective effects of SFPS

Liver injury induced by CCl_4 is the most intensively studied system for xenobiotic-induced oxidative hepatotoxicity (Brautbar & Williams, 2002; Fan et al., 2009; Hwang, Choi, & Jeong, 2009; Shim et al., 2010; Wu, Zhang, Hu, Wu, & Zhao, 2009). In addition, ALT and AST serum enzyme activities have been served as parameters to demonstrate the extent of hepatotoxicity in the mice. In the present study, the effects of SFPS on serum AST and ALT levels are shown in Table 3. Apparently, significant increases in ALT and AST activities ($P < 0.05$) were observed in serums of Group II (model control group) compared with those of Group I (normal control group), indicating that the CCl_4-induced hepatotoxicity in mice was well-established. The increases in serum AST and ALT activities by CCl_4 treatment have been attributed to hepatic structural damage (Recknagel, Glende, Dolak, & Waller, 1989). Moreover, the elevated levels of serum AST and ALT were significantly reduced ($P < 0.05$) in the groups pretreated with SFPS (200 and 400 mg/kg BW).

Table 3
Effects of administration of crude SFPS on the activities of ALT and AST in serum of CCl_4-induced liver injury mice.

Group	AST (U/L)	ALT (U/L)
I (Normal control group)	55.80 ± 4.36[a]	46.88 ± 6.63[a]
II (CCl_4 model control group)	120.30 ± 6.61[b]	91.79 ± 5.08[b]
III (Positive control group)	74.27 ± 2.66[c]	67.85 ± 2.32[c]
IV (SFPS treatment group, 100 mg/kg)	112.00 ± 3.60[b]	89.11 ± 8.36[b]
V (SFPS treatment group, 200 mg/kg)	102.02 ± 2.08[d]	77.93 ± 7.28[d]
VI (SFPS treatment group, 400 mg/kg)	80.07 ± 3.41[e]	60.40 ± 2.41[e]

Different alphabets (a–e) in superscript denote significant difference ($P < 0.05$) according to Dunnet's *t*-test.

Besides AST and ALT, the activities of SOD and CAT and the levels of TAC, GSH and MDA in livers of mice were measured. As shown in Table 4, the activities of antioxidant enzymes (SOD and CAT) and the level of GSH and TAC in livers of model control group mice were significantly decreased compared with those of the control group mice ($P < 0.05$). On the contrary, the level of MDA significantly increased ($P < 0.05$). However, SOD, CAT, GSH and TAC levels were significantly elevated ($P < 0.05$) in the groups by pretreatment with SFPS at a dose of 200 and 400 mg/kg, while the levels of MDA were markedly decreased ($P < 0.05$) in a dose-dependent manner.

It has been reported that CCl_4-induced liver injury is closely associated with the formation of oxidative stress (Hsiao et al., 2003; Hsu et al., 2008; Park, Zhao, Kim, & Sohn, 2005; Wu et al., 2009), and some studies have demonstrated that antioxidant enzymes such as SOD and CAT represent one protection against oxidative tissue-damage (Halliwell & Gutteridge, 1990; Wang, Liu, Tseng, Wu, & Yu, 2004). SOD is an extremely effective antioxidant enzyme. It converts the superoxide anion to H_2O_2 and O_2, thus participating with other antioxidant enzymes in the enzymatic defense against oxygen toxicity. CAT catalyzes the reduction of H_2O_2 to H_2O and O_2, thereby preventing the formation of hydroxyl radicals (Reiter, Tan, Osuna, & Gitto, 2000; Yao et al., 2005). GSH acts as a non-enzymatic antioxidant that reduces H_2O_2, hydroperoxide (ROOH) and xenobiotic toxicity (Kadiiska et al., 2000). The results in the present study showed that the activities of SOD and CAT and the levels of GSH were dramatically decreased in mice by the administration of CCl_4. However, pretreatments with SFPS could improve markedly the activities of those antioxidant enzymes and the levels of GSH in livers of CCl_4-treated mice. The results suggested that the antioxidative system in liver tended to be normalized by the protective action of SFPS.

MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid (Vaca, Wilhelm, & Harms-Ringdahl, 1988). The increase of MDA level in the liver suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant defense mechanisms to prevent the formation of excessive free radicals (Naik, 2003). The data in the present study, demonstrated that the pretreatment of SFPS markedly inhibited the increase of MDA levels in liver of CCl_4-treated mice. In addition, the increase in TAC level demonstrated that the free radicals released in livers were effectively scavenged by the pretreatment of SFPS. Therefore, our results suggested that

Table 4
Effects of SFPS administration on the activities of SOD, CAT and levels of GSH, MDA and TAC in livers of CCl_4-induced liver injury mice.

Group	SOD (U/mg protein)	CAT (U/mg protein)	GSH (mg/g protein)	MDA (nmol/mg protein)	TAC (U/mg protein)
I (Normal control group)	216.08 ± 14.53[a]	245.03 ± 3.16[a]	2.54 ± 0.22[a]	1.86 ± 0.65[a]	2.98 ± 0.75[a]
II (CCl_4 model control group)	124.37 ± 25.06[b]	148.17 ± 2.37[b]	1.39 ± 0.56[b]	7.65 ± 2.8[b]	1.45 ± 0.34[b]
III (Positive control group)	206.4 ± 23.94[a]	214.58 ± 4.56[c]	2.92 ± 0.23[c]	2.77 ± 0.48[c]	3.13 ± 1.07[a]
IV (SFPS treatment group, 100 mg/kg)	169.79 ± 15.77[c]	150.08 ± 2.33[b]	1.87 ± 0.21[b]	5.77 ± 2.38[d]	1.76 ± 0.63[c]
V (SFPS treatment group, 200 mg/kg)	175.89 ± 17.59[d]	171.12 ± 1.36[d]	2.25 ± 0.40[d]	4.81 ± 1.28[e]	2.66 ± 0.61[d]
VI (SFPS treatment group, 400 mg/kg)	196.09 ± 17.08[e]	183.81 ± 4.34[e]	2.32 ± 0.07[d]	3.88 ± 2.56[e]	2.70 ± 0.28[d]

Different alphabets (a–e) in superscript denote significant difference ($P < 0.05$) according to Dunnet's *t*-test.

SFPS had a significant protective effect against CCl_4-induced acute hepatotoxicity in mice and could be explored as novel natural supplement.

The antioxidant properties of polysaccharides are mainly associated with their chemical compositions, molecular weights and conformation (Qi et al., 2005; Sun, Wang, Fang, Gao, & Tan, 2004). It has been reported that polysaccharide with high content of uronic acid exhibits relative higher antioxidant activity (Sun, Liu, & Kennedy, 2010b; Rao & Muralikrishana, 2006). One of the mechanisms involved in antioxidant activity may originated from the hydrogen atom-donating ability of a molecule to a radical, which results in terminating radical chain reactions and converting free radicals to unharmful products (Hu, Zhang, & Kitts, 2000). In addition, polysaccharides with higher ratio of galactose in chemical composition have been reported to have higher antioxidant activity (Capek, Machova, & Turjan, 2009). In the present study, we found that SFPS had a high content of uronic acid and high ratio of galactose in monosaccharide compositions. Thus it seems that these structural properties of SFPS may contribute to its antioxidant activity, and then be related to its hepatoprotective effects. However, the antioxidant mechanisms of polysaccharides are complex and have not been fully characterized. Therefore, the structure–activity relationship of SFPS requires further and deep investigation.

4. Conclusions

Based on the single-factor experiments, BBD was employed to determine the optimal parameters for extraction of SFPS in the present study. Through optimization, a maximum predicted yield of 10.02% was obtained with the following optimized conditions: extracting temperature 94.36 °C, extracting time 4.01 h and ratio of water to material 18.71 mg/ml. Under the optimal conditions of extraction temperature 94 °C, extracting time 4 h and ratio of water to material 19 mg/ml, an actual experimental yield of $9.98 \pm 0.60\%$ was obtained. There was no difference at significant level of 0.05 between the experimental and predicted values. The results suggested that the regression model was accurate and adequate for the extraction of SFPS. Results of GC analysis showed SFPS were composed of rhamnose, arabinose, glucose and galactose in a molar percent of 2.05, 9.16, 28.66 and 60.14, respectively. Furthermore, we demonstrated that the administration of SFPS had a significant protective effect against acute hepatotoxicity induced by CCl_4 in mice.

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