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ORIGINAL ARTICLE

Optimization Extraction Process of Polysaccharides from *Suillus granulatus* and Their Antioxidant and Immunological Activities *In vitro*

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ABSTRACT

Background: *Suillus granulatus* is an edible and medicinal fungus in China. *S. granulatus* polysaccharide (SGP) was considered as the main bioactivity compounds in *S. granulatus*. Therefore, the extraction of SGP and their antioxidant activities were studied in this work. **Materials and Methods:** Fruiting bodies of *S. granulatus* were purchased from a local market (Fushun, China). Response surface methodology was adopted to optimize the extraction conditions of SGP. The antioxidant and immunological activities *in vitro* were also assayed. **Results:** The extraction of SGP was optimized by a Box–Behnken design. The optimal conditions for the extraction of polysaccharides were as follows: Pre-extraction time, 2 h; extraction temperature, 94°C; ratio of water to raw material, 25; and extraction frequency, 2. Under these conditions, the experimental yield of polysaccharides was 5.38% ±0.15%, which agreed with the predicted yield. The antioxidant assay *in vitro* showed that SGPs had relatively high scavenging ability for hydroxyl radicals and higher scavenging ability for 1,1-diphenyl-2-picrylhydrazyl radical. However, the scavenging ability of SGPs for superoxide anion radical and reducing power was relatively low. The polysaccharides also significantly increased splenocyte proliferation *in vitro*. **Conclusion:** SGP possessed good antioxidant and immunological activities *in vitro* and explored as a novel natural antioxidant or functional food.

Key words: Antioxidant, immunological activity, polysaccharides, response surface methodology, *Suillus granulates*

SUMMARY

- The predictive model of *Suillus granulatus* polysaccharide (SGP) extraction is adequate for the extraction process
- SGP possessed a good antioxidant activity *in vitro*
- Lymphocyte proliferation *in vitro* was significantly increased by SGP
- Pictorial abstract (in MS Powerpoint Format) is submitted as a separated file in the online submission system.

Abbreviation used: SGP: *Suillus granulatus* polysaccharides, RSM: Response surface methodology, BBD: Box–Behnken design, Vc: Ascorbic acid, DPPH: 1,1-diphenyl-2-picrylhydrazyl, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ConA: Concanavalin A, LPS: lipopolysaccharide, RPMI-1640: Roswell Park Memorial Institute-1640.

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INTRODUCTION

Mushrooms are appreciated worldwide for their taste and flavor and are consumed both in fresh and processed forms. They are attracting attention as functional health promoters because of their biochemical composition, i.e., their significant contents of proteins, carbohydrates, lipids, enzymes, minerals, and vitamins. Mushrooms polysaccharides are isolated from edible and medicinal fungi, fruiting bodies, mycelia, and fermentation broth. They are called biological response modifiers. Various types of mushroom polysaccharides have been isolated and found to have a wide range of bioactivities, such as antitumor, antioxidant, anticancer, and immunological. Fungal polysaccharides have attracted considerable attention because of their bioactivities, particularly antioxidant activity.[1-3]

Suillus granulatus, which belongs to Eumycota Basidiomycetes, is an edible and medicinal fungus that can be found in the pine forest and

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mixed forest lands in China. However, current studies on *S. granulatus* polysaccharides (SGPs) are limited.

Response surface methodology (RSM) is a statistical method used to solve multivariate problems.[4] With RSM, the number of experiments can be effectively reduced by a reasonable experimental design and multivariate quadratic regression equation to fit the function between factors and response.[5] The regression equations are then analyzed to determine the optimal processing parameters. To date, RSM has been successfully applied to optimize polysaccharide extraction conditions.[6,7]

In this study, a Box–Behnken design (BBD) was designed to optimize the extraction process of SGP, and further assess their biological activities via antioxidant assay and splenocyte proliferation test *in vitro*.

MATERIALS AND METHODS

Materials and chemicals

Fruiting bodies of *S. granulatus* were purchased from a local market (Fushun, China). Ascorbic acid (Vitamin C, Vc), pyrogallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiazolyl blue tetrazolium bromide (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT]), concanavalin A (ConA), and lipopolysaccharide (LPS) were purchased from Sigma Chemicals Co., (St. Louis, MO, USA). Roswell Park Memorial Institute 1640 (RPMI-1640) was purchased from Gibco Invitrogen Co. Fetal calf serum was purchased from Hangzhou Sijiqing Biotech Co., Ltd., (Hangzhou, China). Unless otherwise stated, all chemicals used were analytical grade.

Suillus granulatus polysaccharide extraction

The fruiting bodies of *S. granulatus* (1000 g) were dried in a drying box, homogenized in a grinder, and defatted by ethanol at 80°C for 6 h in a reflux apparatus. The defatted sample was treated with 80% ethanol (v/v) twice to remove some colored materials, monosaccharides, oligosaccharides, and small-molecule materials. The pretreated sample was centrifuged (2000 × *g*, 20 min), and the deposit was vacuum dried for 16 h at 60°C to a constant weight. Each dried pretreated sample (5 g) was extracted with deionized water at a designated extraction frequency, pre-extraction time, temperature, and ratio of water to the raw material. The mixture was centrifuged (2000 × *g*, 20 min), and the supernatant was separated from the insoluble residue with a four-layer filter cloth. The extract was precipitated by adding ethanol to a final concentration of 75% (v/v) and then incubated overnight. The precipitated polysaccharides were collected via centrifugation (2000 × *g*, 20 min) and deproteinized via the proteinase–Sevag method.[8] The supernatant was placed in a dialysis bag for 2 days to remove small protein molecules and some impurity ions after deproteinization. The supernatant was then lyophilized to obtain SGP. The Bradford method was used to determine the protein content of the polysaccharides using bovine serum albumin as the standard.[8] The Bradfordmethod was used to determine the protein content of polysaccharides using BSA as standard.[9] The polysaccharide content was measured using the phenol–sulfuric acid method with D-glucose as the standard.[10]

Experimental design and statistical analysis

After determining the preliminary range of extraction variables through a single-factor test, a BBD with three independent variables (X_1, pre-extraction time; X_2, extraction temperature; and X_3, ratio of water to raw material) at three levels was performed. For statistical calculations, the variables were coded as:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

Where x_i is the coded value of the independent variable, X_i is the actual value of the independent variable, X_0 is the actual value of X_i at the center point, and ΔX_i is the value of step change. The ranges of independent variables and the levels that were selected on the basis of the results of single-factor analysis are presented in Table 1.

Table 1: Experimental domain of Box–Behnken design

Variables	Levels		
	−1	0	1
Extraction time (X_1) (h)	2	3	4
Extraction temperature (X_2) (°C)	80	90	100
Ratio of water to raw material (X_3) (n)	15	20	25

The design consisted of 17 experimental points, each of which was performed in triplicate in a randomized order. The response value in each trial was the average of the duplicates. The BBD data were analyzed using multiple regression analysis to fit the following quadratic polynomial model:

$$Y_k = \beta_{k_0} + \sum_{i=1}^{4} \beta_{k_i} x_i + \sum_{i=1}^{4} \beta_{k_{ii}} x_i^2 + \sum_{i<j=2}^{4} \beta_{k_{ij}} x_i x_j \quad (2)$$

Where Y_k is the response function, and β_{k0} is an intercept. β_{ki}, β_{kii}, and β_{kij} are the coefficients of the linear, quadratic, and interactive terms, respectively. x_i and x_j represent the coded independent variables. The fitted polynomial equation was expressed using surface and contour plots to visualize the relationship between the response and experimental levels of each factor and then deduce the optimal conditions. The regression coefficients of individual linear, quadratic, and interaction terms were determined through variable analysis. The regression coefficients were used to generate three-dimensional (3D) surface plots and contour plots from the fitted polynomial equation via statistical calculation. Design-Expert software (version 8.0.6, Stat-Ease Inc., Minneapolis,MN, USA) was used to analyze the experimental data. Statistical significance was considered at $P < 0.05$.

Antioxidant activity assay

Superoxide radical scavenging assay

Superoxide anion radical scavenging activity was measured as described by Wang *et al.*[11] A Tris solution (100 mM) was prepared, and concentrated HCl was added dropwise to adjust the pH to 8.2 and form Tris–HCl buffer. Approximately 4.5 mL of the Tris–HCl buffer solution was mixed with 4.2 mL of Milli-Q H_2O in a test tube and then incubated for 20 min on a 25°C water bath. Simultaneously, the sample and 25 mM pyrogallol solution were preheated on a 25°C water bath. After 20 min of incubation, 1 mL of the polysaccharide solution (125–3750 mg/L) and 0.3 mL of pyrogallic acid were added and mixed quickly. The resulting mixture was incubated at a constant temperature bath of 25°C for 5 min. Finally, 1 mL of 8 mM HCl was added to the mixture to terminate the reaction. The absorbance of the mixture was measured at 325 nm using Milli-Q H_2O as the zeroing tube and Vc as the positive control. Each sample concentration was analyzed in three parallel experiments. The blank tube contained Milli-Q H_2O instead of the sample, and the sample tube was treated as described above. The superoxide anion radical scavenging activity of the polysaccharides was calculated as:

$$\text{Scavenging rate (\%)} = (A_0 - [A_i - A_{i0}])/A_0 \times 100 \quad (3)$$

Where A_0 is the absorbance of the blank, A_i is the absorbance of the polysaccharides or Vc, and A_{i0} is the absorbance of the polysaccharide solution itself.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was determined as described by Winterbourn and Sutton.[12] The reaction mixture contained 1 mL of 9 mM salicylic acid ethanol solution, 1 mL of 9 mM $FeSO_4$, 1 mL of the polysaccharide solution (50–2500 mg/L), and 1 mL of 9 mM H_2O_2. After incubation on a 37°C water bath for 30 min, the absorbance was

measured at 510 nm using Milli-Q H_2O as the zeroing tube and Vc as the positive control. The blank tube contained Milli-Q H_2O instead of the sample, and the remaining operations referred to the sample tube. The EC_{50} value (mg/L) of the polysaccharides or Vc was the effective concentration at which 50% of the hydroxyl radicals were scavenged. The hydroxyl radical scavenging activity of the sample was evaluated using the following formula:

$$\text{Scavenging rate (\%)} = (A_0 - [A_i - A_{i0}])/A_0 \times 100 \quad (4)$$

where A_0 is the absorbance of the blank, A_i is the absorbance of the polysaccharides or Vc sample, and A_{i0} is the absorbance of the polysaccharide solution without H_2O_2.

1,1-diphenyl-2-picrylhydrazyl scavenging assay

DPPH radical scavenging activity was evaluated in accordance with the method described by Shimada *et al.*[13] with slight modifications. The reaction mixture contained 2 mL of DPPH (0.2 mM in 95% ethanol), 1 mL of Milli-Q H_2O, and 1 mL of the polysaccharide solution (125–3750 mg/L). The mixture was incubated on a 25°C water bath for 30 min, and the absorbance of the mixture was determined at 517 nm using Milli-Q H_2O as the zeroing tube and Vc as the positive control. The EC_{50} value (mg/L) of the polysaccharides or Vc was the effective concentration at which 50% of the DPPH radicals was scavenged. The antioxidant activity of the sample was calculated as follows:

$$\text{Scavenging rate (\%)} = (A_0 - [A_i - A_{i0}])/A_0 \times 100 \quad (5)$$

Where A_0 is the absorbance of the blank, A_i is the absorbance of the polysaccharides or Vc sample, and A_{i0} is the absorbance of the polysaccharide solution without DPPH.

Reducing power

The reducing power of the polysaccharides was determined as described by Deng *et al.*[13,14] The reaction mixture contained 2.5 mL of phosphate buffer (pH 6.6, 0.2 M), 2.5 mL of potassium ferricyanide (1%, w/v), and 2 mL of the polysaccharide solution (125–3750 mg/L). After incubation on a 50°C water bath for 30 min, the mixture was added with 2.5 mL of trichloroacetic acid (10%, w/v) to terminate the reaction and then centrifuged (1200 × *g*, 10 min). Approximately 2.5 mL of the supernatant was collected and mixed with 2.5 mL of Milli-Q H_2O and 0.5 mL of $FeCl_3$ (0.1%, w/v). After incubation at room temperature for 15 min, the absorbance of the polysaccharides was measured at 700 nm using Milli-Q H_2O as the blank and Vc as the positive control.

Splenocyte cell proliferation assay

Cell proliferation was assessed using the MTT-based colorimetric assay. 10 BALB/c mice (male, 8 weeks to 12 weeks old) were sacrificed by cervical dislocation, and their spleens were aseptically removed. Spleen cells were obtained by gently placing the organ in RPMI-1640 medium under aseptic conditions followed by centrifugation at 3000 × *g* for 10 min at room temperature. The red blood cells were removed using hemolytic Gey's solution. After washes twice, the cells were resuspended in RPMI-1640 complete medium. The cell concentration was adjusted to 2×10^6 cells/mL, and the cell suspension was plated on a 96-well culture plate with or without ConA (5.0 μg/mL) or LPS (20.0 μg/mL). SGP (at 50, 100, 200, 400, and 800 μg/mL) were added to each cell. After incubation for 72 h at 37°C in a humidified 5% CO_2 incubator, each well was pulsed with MTT. The plate was further incubated for another 4 h. After aspirating the supernatant from the wells, 100 μL of dimethylsulfoxide was added to dissolve the formazan crystals. The absorbance of each well was read at 570 nm using a microplate reader (Model 680, Bio-Rad Co., USA).

Statistical analysis

The data were presented as mean ± standard error of mean. Statistical analyses were performed using Student's *t*-test and one-way analysis of variance. All computations were done by employing the statistical software (SPSS, version 13.0, SPSS Inc., Chicago, IL, USA). A significant difference between two groups was defined as $P < 0.05$.

RESULTS AND DISCUSSION

Single factor experiments

Effect of extraction frequency on Suillus granulatus polysaccharide yield

The effect of extraction frequency on SGP yield is shown in Figure 1a. Basing from the principle of a single variable, we tested different extraction frequencies while keeping the other extraction conditions constant as follows: Pre-extraction time, 2 h; extraction temperature, 100°C; and ratio of water to raw material, 20. The extraction yield of the SGP growth rate increased as the extraction frequency was increased from 1 to 2. However, the effect of increasing extraction efficiency on growth rate was unclear. Considering the cost, we tentatively decided the extraction frequency to be 2 in subsequent trials.

Effect of pre-extraction time on Suillus granulatus polysaccharide yield

Figure 1b shows the effect of pre-extraction time on SGP yield. Basing from the principle of a single variable, we selected the other extraction conditions as follows: Extraction frequency, 2; extraction temperature, 100°C; and ratio of water to raw material, 20. As shown in Figure 1b, the maximum extraction yield was reached at about 2 h. After 2 h, SGP yield decreased with increasing time. Considering that prolonged high temperatures promote the hydrolysis of polysaccharides, we determined the appropriate pre-extraction time to be 2 h in subsequent experiments.

Effect of extraction temperature on Suillus granulatus polysaccharide yield

The extraction coefficient increases with increasing temperature because of the heightened solubility of polysaccharides.[15] In this study, extraction was conducted at different temperatures under the following extraction parameters: Extraction frequency, 2; pre-extraction time, 2 h; and ratio of water to raw material, 20. As shown in Figure 1c, SGP yield increased with increasing extraction temperature from 70°C to 90°C and then peaked at 90°C. SGP yield declined as the temperature exceeded 90°C; this result can be ascribed to the fact that a high temperature destroys the molecular structure of polysaccharides. Thus, the extraction temperature of 90°C was selected for subsequent experiments.

Effect of ratio of water to raw material on Suillus granulatus polysaccharide yield

SGP yield was affected by different ratios of water to raw material. To study the effect of different ratios of water to raw material on SGP yield under the principle of a single variable, the extraction frequency, pre-extraction time, and extraction temperature were fixed at 2, 2 h, and 90°C, respectively. As shown in Figure 1d, SGP yield increased as the ratio of water to raw material increased from 15 to 20 and then peaked at 20. However, the extraction yield of SGP was only slightly affected when the ratio of water to raw material ranged from 20 to 35.

Basing from the above single-factor experiments, we adopted the following conditions in the RSM experiments: Pre-extraction time, 2–4 h; extraction temperature, 80–100°C; and ratio of water to raw material, 15–25.

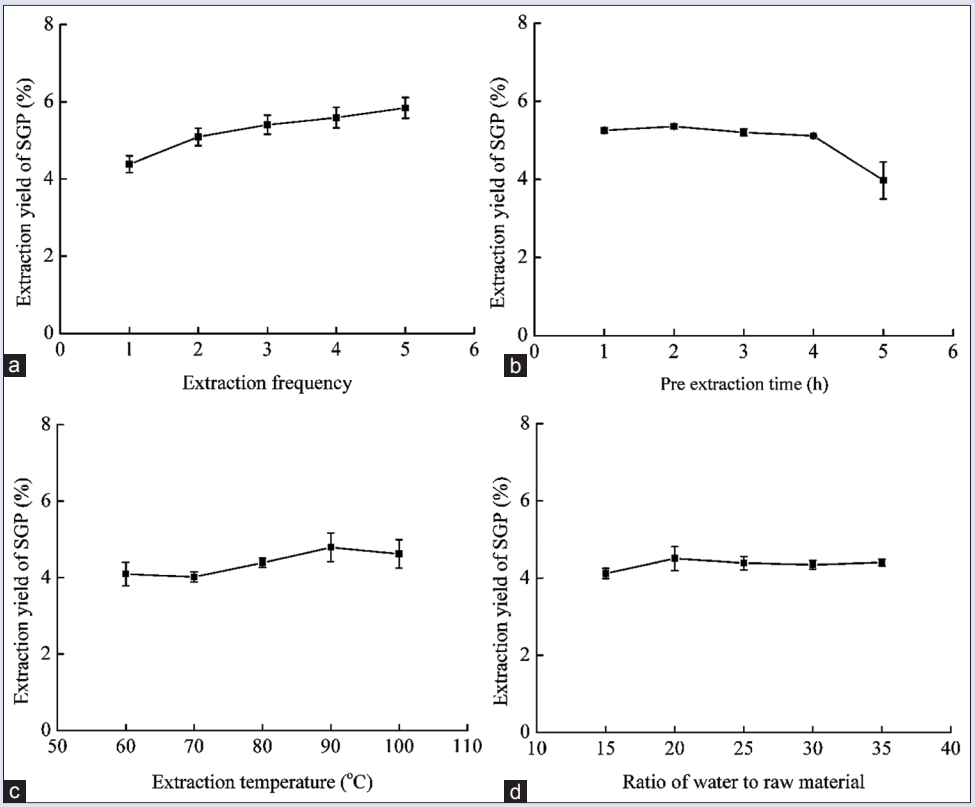

Figure 1: Effect of extraction parameters on *Suillus granulatus* polysaccharides: (a) Extraction frequency; (b) pre-extraction time; (c) extraction temperature; (d) ratio of water to raw material

Table 2: Response surface central composite design and results for yield of Suillus granulatus polysaccharide

X_1, extraction time (h)	X_2, extraction temperature (°C)	X_3, ratio of water to raw material	Yield of SGP (%)
−1	−1	0	3.98
1	−1	0	3.59
−1	1	0	4.88
1	1	0	4.20
−1	0	−1	4.90
1	0	−1	5.15
−1	0	1	5.07
1	0	1	5.00
0	−1	−1	4.06
0	1	−1	4.81
0	−1	1	3.58
0	1	1	4.86
0	0	0	5.08
0	0	0	5.05
0	0	0	5.16
0	0	0	5.15
0	0	0	4.96

SGP: *Suillus granulatus* polysaccharide

Statistical analysis and model fitting

The design matrix and corresponding results of RSM experiments, which determined the effects of the three independent variables X_1, X_2, and X_3, are listed in Table 2. The maximum SGP yield was recorded under the following experimental conditions: Extraction frequency, 2; pre-extraction time, 2 h; extraction temperature, 94°C; and ratio of water to raw material, 25. Multiple regression analysis of the experimental data revealed that the relationship between the response and test variables can be described by following multivariate quadratic regression equation:

$$Y = 5.08 - 0.11X_1 + 0.44X_2 - 0.050X_3 - 0.074X_1X_2 - 0.080X_1X_3 + 0.13X_2X_3 - 0.11X_1^2 - 0.81X_2^2 + 0.058X_3^2 \quad (6)$$

The fitting statistics of the extraction yield Y of the selected quadratic predictive model are shown in Table 3. The determination coefficient determined by ANOVA of the quadratic regression model was $R^2 = 0.9422$. This value indicates that only 5.78% of the total variations could not be explained by the model. The adjusted determination coefficient also confirmed that the model was highly significant. Simultaneously, the low coefficient of variation (4.32) indicates that the experimental values have a high degree of accuracy and reliability. The model was demonstrated to be adequate for prediction within the range of experimental variables.

The model F-value of 12.68 implies that the model is significant [Table 3]. The probability that a "model F-value" this large could occur because of noise was only 0.15%. Values of "Prob >F" <0.0500 indicate that the model terms are significant.[16] In this case, X_2 and were significant model terms. The "lack of fit F-value" of 13.24 implies that the lack of fit is significant. The probability that a "lack of fit F-value" this large could occur because of noise was only 1.52%.

Optimization of *Suillus granulatus* polysaccharide extraction conditions

Design-Expert software (version 8.0.6) was used to obtain the 3D response surface and contour plots of the BBD. The results of SGP yield based on the pre-extraction time (X_1), extraction temperature (X_2), and ratio of water to raw material (X_3) are shown in Figure 2. The main goal of RSM is to efficiently identify the optimal values of independent variables

for maximizing responses.[17] In the response surface and contour plots, the SGP yield was obtained using two continuous variables while fixing the third variable at its respective zero level (center value of the testing ranges). The maximum predicted value quantified by the surface plot was confined to the smallest ellipse in the contour diagram. Elliptical contours were obtained when a perfect interaction existed among the independent variables. The independent variables and maximum predicted values corresponded to the optimal values of the dependent variables (responses) obtained from the equations.

The 3D surface and contour plots that were constructed on the basis of the independent variables (preextraction time and extraction temperature) are shown in Figure 2a and d. The third independent variable (ratio of water to raw material) was maintained at zero level. The maximum SGP yield was achieved when the preextraction time was 2 h and extraction temperature was 94°C. The effects of different extraction times and ratios of water to raw material on SGP yield when the extraction temperature was fixed at zero level are shown in Figure 2b and e. The maximum SGP yield was obtained when the preextraction time and ratio of water to raw material were set to 2 h and 25, respectively. Figure 2c and f show the SGP yield at varying extraction temperatures and ratios of water to raw material when the pre-extraction time was fixed at zero level. The yield increased as the extraction temperature was increased from 80°C to 94°C and decreased beyond 94°C. The yield also increased as the ratio of water to raw material was increased from 15 to 25 and declined from 25 to 30.

Figure 2 shows that the optimal extraction conditions of SGP were as follows: Pre-extraction time, 2 h; extraction temperature, 94°C; ratio of water to raw material, 25; and extraction frequency, 2. Among the three extraction parameters, extraction temperature and pre-extraction time exerted the most significant effects on SGP yield.

Table 3: Analysis of variance for the fitted quadratic polynomial model

Source	SS	df	MS	*F*	*P* > *F*
Model	4.65	9	0.52	12.68	0.0015
Residual	0.29	7	0.041		
Lack of fit	0.26	3	0.086	13.24	0.0152
Pure error	0.026	4	0.0065		
Cor total	4.94	16			
	R^2=0.9422	R^2_{adj} =0.8679	CV=4.32		

SS: Sum of square; MS: Mean square; CV: Coefficient of variation

Verification of the predictive model

The suitability of the model equations for predicting the optimal response values was determined under the optimized conditions described above. Compared with the predicted SGP yield (5.30%), the mean value of

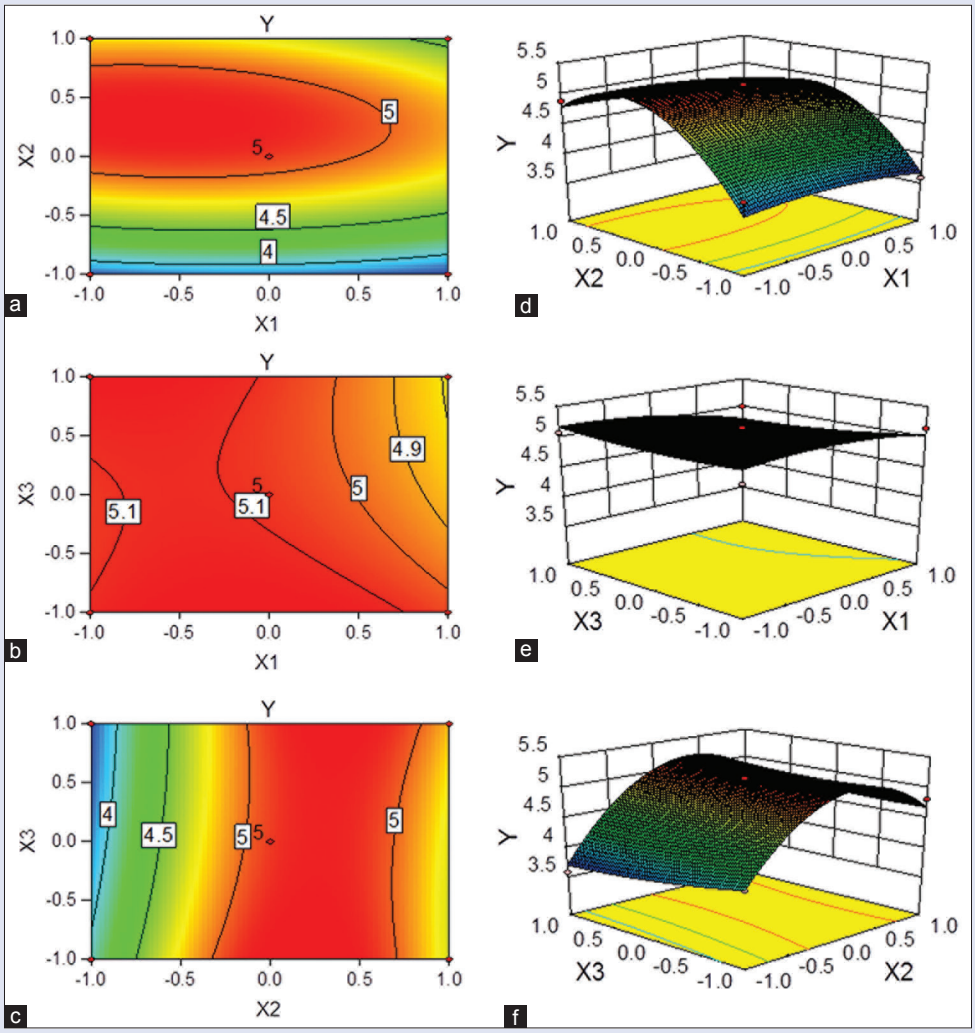

Figure 2: Contour plots (a-c) and three-dimensional response surface plots (d-f) showing the effects of variables (X_1: pre-extraction time; X_2: extraction temperature; X_3: ratio of water to raw material) on the response *Y* (yield of *Suillus granulatus* polysaccharides)

5.38% ±0.15% obtained from the experiments indicates the validity of the RSM model and shows that the model is suitable for extraction.

Antioxidant activity

The antioxidant activities of compounds are attributed to various reactions and mechanisms, such as radical scavenging, prevention of chain initiation, reductive capacity, and binding of transition metal ion catalysts.[18] In the present study, the anti-oxidative activities of SGP *in vitro* were evaluated by assessing the reducing power and scavenging abilities for superoxide anions, hydroxyls, and DPPH radicals., the protein content of SGP was assayed before antioxidant activity and SGP shows there only has <1% protein in the nucleosome core particle (NCP). It means most of the protein removed from NCP.

The superoxide anion, one of the precursors of singlet oxygen and hydroxyl radicals, indirectly initiates lipid peroxidation. Superoxide anions can produce various free radicals and oxidizing agents, so they can destroy cell.[19] The results of superoxide radical scavenging assay are shown in Figure 3a. The scavenging rate of Vc for superoxide anion radical was directly proportional to its concentration, and the EC_{50} value of Vc was 576.8 mg/L. The superoxide radical scavenging rate of SGP was relatively low, and its EC_{50} value was not assayed within the experimental range.

Hydroxyl radicals can easily pass through cell membranes; quickly react with many intracellular biomolecules, and cause tissue damage and even cell death. Therefore, removing hydroxyl radicals is important for the protection of living systems.[20] Figure 3b shows that the maximum hydroxyl radical scavenging rate of SGP was about 58% at 2000 mg/L, and its EC_{50} was about 994.2 mg/L, which was within the experimental range. In this concentration, the scavenging rate of Vc reached nearly 100%, and its EC_{50} was about 285.3 mg/L.

The DPPH scavenging assay is widely used for evaluating the free radical scavenging activities of natural compounds.[21] The scavenging effects of SGP on DPPH radicals are shown in Figure 3c. Both SGP and Vc showed evident scavenging activity for DPPH radicals in a concentration-dependent manner within the concentration range of 50–500 mg/L. The scavenging activity of SGP and Vc growth rate were relatively small within a relatively high concentration range of 500–1500 mg/L. With the increase in the concentration of samples, the scavenging rate of SGP reached 100%. The EC_{50} value of SGP was 354.8 mg/L, and that of Vc was approximately 36.9 mg/L.

The reducing powers of SGP and Vc are shown in Figure 3d. Compared with Vc, the reducing capacity of SGP was relatively low.

Lymphocyte proliferation activity

Lymphocytes proliferation is an indicator of immune activation. Lymphocytes induced by ConA *in vitro* may be used as a method to evaluate T lymphocyte activity while that induced by LPS may be used to evaluate B lymphocyte activity. Fungal polysaccharides inhibit tumor by regulating immune system primarily. They can activate T lymphocyte and B lymphocyte to show their effects on the immune system.[22,23] In this work, SGP was subjected to immune tests to evaluate its effects on lymphocyte proliferation. As shown in Figure 4a, SGP significantly increased lymphocyte proliferation ($P < 0.05$) when it concentration at 100 μg/mL or more. However, SGP has no synergy with ConA or LPS within the test dosage range [Figure 4b and c].

CONCLUSION

This study examined the extraction conditions of SGP and further analyzed their antioxidant and immunological activities *in vitro*. Combined with single-factor experiment and response surface analysis, the optimal extraction conditions for the production of SGP were determined. Antioxidant experiments proved that SGP exerted certain

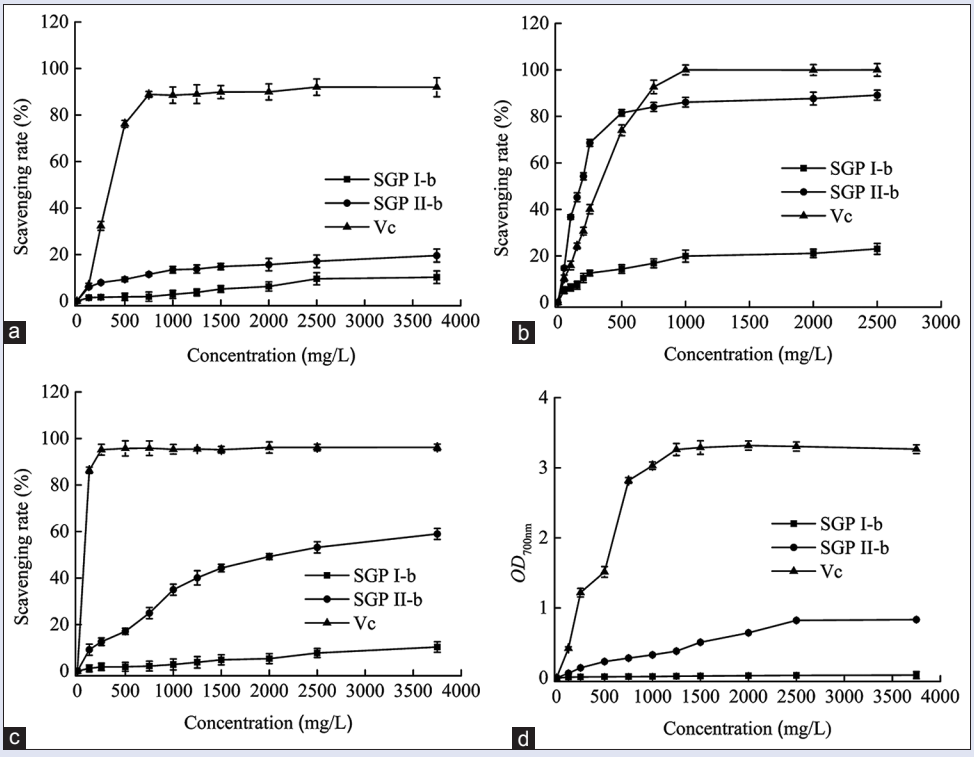

Figure 3: Antioxidant activity assay of *Suillus granulatus* polysaccharides and Vc. (a) Scavenging effects on superoxide anion radicals; (b) scavenging effects on hydroxyl radicals; (c) scavenging effects on 1,1-diphenyl-2-picrylhydrazyl; (d) reducing power of *Suillus granulatus* polysaccharides and Vc

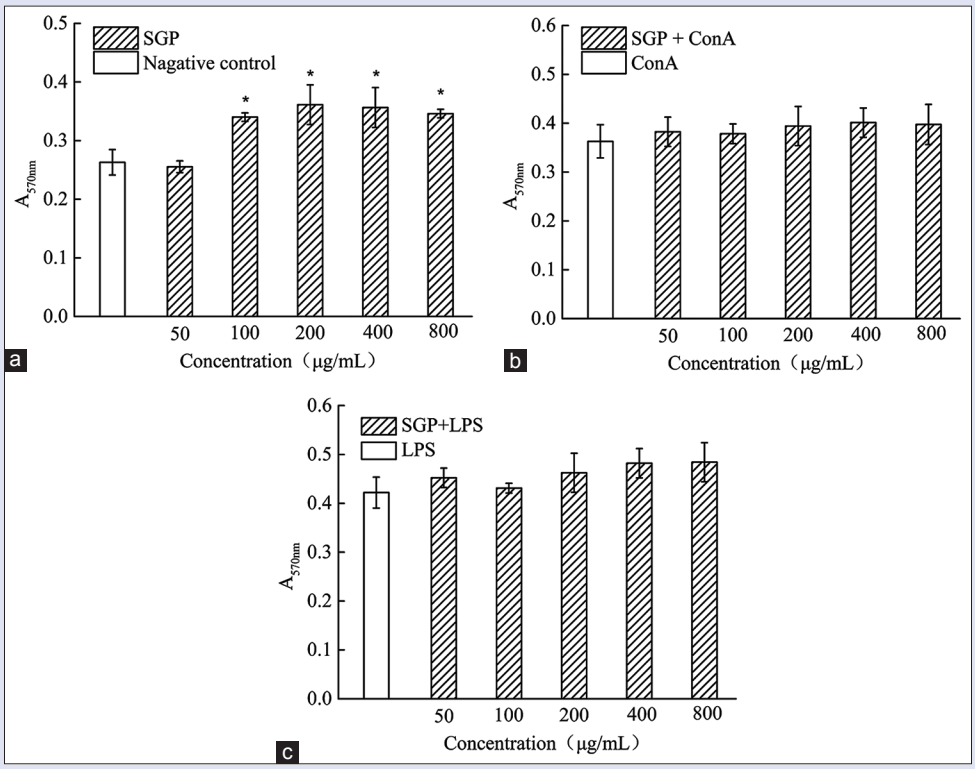

Figure 4: Effect of *Suillus granulatus* polysaccharides with or without concanavalin A-induced and lipopolysaccharide-induced on splenocyte proliferation (a) *Suillus granulatus* polysaccharides, values are mean ± standard deviation, **P* < 0.05, ***P* < 0.01 vs. negative control; (b) *Suillus granulatus* polysaccharides + concanavalin A, values are mean ± standard deviation, **P* < 0.05, ***P* < 0.01 vs. concanavalin A; (c) *Suillus granulatus* polysaccharides + lipopolysaccharide, values are mean ± standard deviation, **P* < 0.05, ***P* < 0.01 vs. lipopolysaccharide)

antioxidant effects. SGP also has a significant lymphocyte proliferation activity. The results of this study could facilitate the development of wild resources in the local region, and increase the edible and medicinal value of *S. granulatus*. In-depth research on the polysaccharide structure and bioactivities is currently underway in our laboratory. Therefore, further characterization and applications of SGP in functional medicine are expected.

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Conflicts of interest

There are no conflicts of interest.

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