



Optimization extraction and bioactivities of polysaccharide from wild *Russula griseocarnosa*



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ABSTRACT

The extraction conditions and biological activities of polysaccharides from wild *Russula griseocarnosa* (PRG) were investigated. Response Surface Methodology (RSM) with a Box-Behnken Design (BBD) was used to optimize extraction conditions. The optimal extraction parameters of PRG were as follows: extracting time 4 h, extraction temperature 77.3 °C and liquid-solid ratio 42.5 g/L. Furthermore, the data demonstrated that PRG exhibited antioxidant activities evidenced by reducing power to scavenge the DPPH, ABTS, hydroxyl radical and superoxide radical. PRG showed the activity of anti-cervical carcinoma cells Hela and Siha. In conclusion this study offered an efficient extraction method of wild *Russula griseocarnosa* polysaccharide, and the results suggested PRG had good antioxidant and inhibitory activities against cervical carcinoma cells, and PRG could be developed as a novel natural functional food.

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1. Introduction

Polysaccharides are widely distributed in animal, plants and microorganisms, which is a large natural resource (Tao et al., 2016; Ma et al., 2013; Atta et al., 2017). Recently, considerable interest has arisen in characterizing the polysaccharide of mushrooms, which show various pharmacological functions, including anti-tumor, immunoregulatory and antioxidant activities (Du et al., 2016; Palanisamy et al., 2016; Tian et al., 2016; Sun et al., 2013). Polysaccharides from *Agrocybe cylindracea* SL-02 had enhancement effects on the reducing power of reactive oxygen species (Liu et al., 2016b). Additionally, polysaccharides from edible mushroom *Agaricus bisporus* (Lange) Sing. Chaidm was effective in scavenging ability on DPPH and hydroxyl radicals (Liu et al., 2016a; Li et al., 2015).

Many studies have focused on the anti-tumor activities of polysaccharide. A neutral polysaccharide from *Letinus giganteus* had anti-proliferation of HepG2 cells via intrinsic mitochondrial apoptosis and PI3K/Akt signaling pathway (Chen et al., 2016; Tian et al., 2016; Halim et al., 2017; Muhammad et al., 2017).

It is widely recognized that natural mushroom polysaccharides has tremendous potential for promoting human health. Efficient extraction techniques of polysaccharide are important for enlarging their application in the practical production. RSM was recognized a useful method combined with empirical and statistical characterization, which was widely employed in the commercial application for extraction conditions optimization in the process. It is an efficient method for improving and optimizing industrial processes (Sarfraz et al., 2016; Zheng et al., 2016). As known, the main advantages of RSM are to evaluate multiple factors and the interactions through less experimental trials (Wu et al., 2013; Ishaq and Jafri, 2017).

Russula griseocarnosa was originally discovered as a new species in China in 2009. The reports of bioactivity on *R. Griseocarnosa* remain less. In order to explore and use the polysaccharide from wild *R. griseocarnosa*, RSM combined with a BBD was employed to obtain the optimal polysaccharide extraction parameters in this paper. Then, the antioxidant and anti-tumor activities of PRG were studied.

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2. Materials and methods

2.1. The chemical reagents

The dry *R. griseocarnosa* sporocarp was purchased from Fujian, Southeast of China. The material was identified by Professor Bau Tolgor of Jilin agriculture university, Changchun, China.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from sigma Co. The Fetal bovine serum (FBS) and DMEM medium were purchased from Gibco, Biomyx was purchased from Biological Industries. CCK8 kit was purchased from TransGen Biotech. Other chemicals including anhydrous ethanol, petroleum ether, phenol, acetone, sulfuric acid, potassium persulfate, H₂O₂, Trolox, Vitamin C and salicylic acid-ethyl alcohol were purchased locally and were of analytical grade.

2.2. Extraction of polysaccharide

Dry *R. griseocarnosa* sporocarp were firstly grinded into powder, and filtered with 40 mesh sieved. The polysaccharides of *R. griseocarnosa* were extracted as reported method with minor modifications.

Briefly, the dry filtered powder was pretreated with petroleum ether and 95% ethanol to remove colored materials and other low molecular chemicals. Then the powder were dried and extracted under the conditions with designed factors and their levels. The crude polysaccharides solution was concentrated, and resuspended with a three volumes of anhydrous ethanol and incubated. After 12 h, the crude polysaccharide solution with anhydrous ethanol were centrifuged at 3000g for 20 min, then the pellets were kept and washed with absolute ethanol and acetone. Finally, the precipitates were dried.

Table 1
The three variables and levels in quadratic orthogonal rotation combination design.

Independent variables	Symbol	Levels		
		−1	0	1
Time (h)	X ₁	2	3	4
Temperature (°C)	X ₂	70	80	90
Liquid-solid ratio (g/L)	X ₃	30	40	50

Table 2
Box-Behnken design and results for the yield.

Run	Coded variable levels			Experimental values (%)	Predicted values (%)
	X ₁	X ₂	X ₃		
1	−1.00	0.00	−1.00	4.21	4.28
2	0.00	0.00	0.00	5.48	5.36
3	−1.00	0.00	1.00	4.27	4.27
4	0.00	0.00	0.00	5.16	5.36
5	0.00	1.00	1.00	3.95	4.08
6	1.00	1.00	0.00	4.54	4.48
7	0.00	1.00	−1.00	4.46	4.52
8	0.00	0.00	0.00	5.30	5.36
9	0.00	0.00	0.00	5.43	5.36
10	1.00	0.00	−1.00	4.41	4.41
11	0.00	−1.00	−1.00	3.44	3.31
12	0.00	−1.00	1.00	4.30	4.24
13	1.00	−1.00	0.00	4.86	4.99
14	−1.00	−1.00	0.00	3.51	3.57
15	1.00	0.00	1.00	5.00	4.93
16	−1.00	1.00	0.00	5.25	5.12
17	0.00	0.00	0.00	5.43	5.36

2.3. Single factor design for polysaccharide of *R. Griseocarnosa* sporocarp

The range of 3 designed variables was firstly confirmed by the Single-Factor Design. The three key factors consisted of extraction time (X₁, 1, 2, 3, 4 and 5 h), extraction temperature (X₂, 50, 60, 70, 80 and 90 °C) and liquid-solid ratio (X₃, 20, 30, 40, 50 and 60 g/L). One factor changed according to the design while the other two factors kept unchanged, and the polysaccharide of *R. Griseocarnosa* (PRG) sporocarp were extracted as described in Section 2.2.

2.4. Experimental design of RSM

RSM was applied to predict the optimal extraction conditions of PRG according to the data of single-factor experiment. Then the Design Expert Software (8.0.6) was applied to the experiment design, data analysis and model building. The Box-Behnken Design (BBD) was used to explore the optimal levels of the 3 factors (X₁, X₂ and X₃) as shown in Table 1. Every factor was designed into three levels, and coded as −1, 0, +1 for low, intermediate and high levels, respectively. For statistical calculation, the 3 variables were coded as follows:

$$xi = \frac{(Xi - X0)}{\Delta Xi}$$

In the equation, X_i is the real value of factor, X₀ is the real value of the X_i at the center point, ΔX_i is the step change value in X_i, x_i is the coded value of the factor, and i = 1, 2, 3.

This BBD comprising of 17 experimental runs was finished in a random order in Table 2. There were 12 factorial points and 5 axial points which were used to allow for estimation of a pure error sum of squares, the experimental data were fitted to the following quadratic polynomial model:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j>1}^3 \beta_{ij} X_i X_j$$

Y denotes the response values, β₀, β_i, β_{ij} and β_{ii} are the constant coefficient, the linear coefficient, the interaction coefficients and the squared coefficients, respectively. X_i and X_j are the coded independent variables, X_iX_j and X_i² represent the interaction and quadratic terms.

The result analysis was finished with Design Expert software (8.0.6) and ANOVA. The fitness of the polynomial model equation was expressed by the coefficient of determination R² and the

values of adjusted R^2 of models. The P -values of <0.05 were considered statistically significant. The significance of the regression coefficient was tested by F -test as well. The regression coefficients were then used to make statistical calculation to generate contour and dimensional maps from the regression models.

2.5. Measurement of the yield

The total sugar was determined by phenol-sulfuric acid method as described in previous report with minor modifications (Siu et al., 2016). The total sugar yield (% w/w) was calculated as the total sugar content of extraction divided by dried power weight, and the equation is as follow:

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

where W_1 was the weights (g) of crude PRG, and W_0 represents the powder weight (g).

2.6. Antioxidant activity

2.6.1. DPPH scavenging assay

DPPH scavenging activity was determined as previous report (C. X. Yang et al., 2013; X.B. Yang et al., 2013). Briefly, DPPH dissolved in 0.2 mM anhydrous ethanol was prepared, and the solution (1.0 mL) was added to 3.0 mL PRG solution with various concentrations (1, 2, 3, 4, 5 and 6 mg/mL). The reaction mixture was kept in the dark for 30 min at room temperature. Finally, A_{517} was recorded using a spectrophotometer. Vitamin C (Vc) was considered as the positive control. Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. The DPPH scavenging activity (RSA) was calculated by using the following equation:

$$\text{RSA (\%)} = \frac{A_c - A_s}{A_c} \times 100\%$$

where A_c denotes the absorbance of DPPH dissolved in anhydrous ethanol, A_s is the absorbance of PRG with DPPH solution.

2.6.2. ABTS assay

The antioxidant activity with ABTS was determined as described previously (Re et al., 1999). The ABTS radicals were prepared by adding 2.6 mM potassium persulfate to a 7.4 mM ABTS solution in PBS buffer (pH 7.4). Then the ABTS radicals was kept at room temperature in darkness for 16 h. Before use, the ABTS stock solution was adjusted to A_{734} at 0.7 with PBS. 2.0 mL of ABTS solution was added to PRG with a series of concentrations (1, 2, 3, 4, 5 and 6 mg/mL), and the reaction mixture was kept at room temperature. After 20 min, A_{734} was immediately measured. Vc was considered as the positive control. The RSA was also calculated using equation:

$$\text{RSA (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\%$$

A_0 denotes the absorbance of ABTS without the PRG solution (water instead of PRG). A_1 denotes the absorbance of PRG solution and ABTS, and A_2 denotes the absorbance of the sample with deionized water.

2.6.3. Hydroxyl radical assay

The hydroxyl radical scavenging activity was measured as described report (Smirnoff and Cumbes, 1989). The hydroxyl radical was produced by Fenton reaction. The reaction contains 8.8 mM H_2O_2 (5 mL), 9 mM salicylic acid-ethyl alcol (5 mL) and 4 mL PRG solution with a series of concentrations (1, 2, 3, 4, 5 and 6 mg/mL). Then the reaction was started by 0.5 mL of 9 mM H_2O_2 , and

incubated for 30 min at 37 °C, then A_{510} was read. Trolox was considered as the positive control.

The RSA was obtained by using the following equation:

$$\text{RSA (\%)} = \frac{A_0 - (A_i - A_j)}{A_0} \times 100\%$$

A_0 denotes the absorbance of the negative control (water instead of the PRG solution); A_i , absorbance of the reaction with PRG solution; A_2 , absorbance of the reaction without H_2O_2 .

2.6.4. Superoxide radical assay

The modified PMS-NADH system was employed to measure the capacity of PRG to inhibit the photochemical decrease of nitroblue-tetrazolium (NBT) (Iftakhar et al., 2015; C.X. Yang et al., 2013; X.B. Yang et al., 2013). Briefly, the various concentrations (1, 2, 3, 4, 5 and 6 mg/mL) of the PRG solutions were added to 1.0 mL NBT, 1.0 mL NADH and 0.4 mL PMS. Then the reaction mixture was kept for 5 min after mixing, and then the A_{560} was recorded. Ascorbic acid was considered as standard. The RSA was obtained as follows:

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

2.7. Cell culture and cell viability analysis

Hela and Siha cells were purchased from ATCC and cultured with DMEM medium with 10% FBS, 1% biomyces in a cell incubator with 5% CO_2 at 37 °C. 100 μ L in logarithmic phase cells (1×10^5 /mL) were plated into 96-well plate and incubated at 37 °C overnight. Then the cells were treated with various concentrations PRG (25, 50, 75, 100, 125 and 150 μ g/mL) for 24 h. The supernatants were removed, and 100 μ L CCK8 solution (10 μ L CCK8:100 μ L medium) was added to each well. The Cells were incubated in the darkness for 1 h, and the absorbance was detected by micro-plate at 450 nm.

2.8. Statistical analysis

Data were analyzed by ANOVA and expressed as mean \pm standard deviation (SD) for three replicates. Significance at $p < 0.05$ levels was determined by ANOVA followed by Duncan's multiple comparison tests.

3. Results

3.1. Single factor result analysis

3.1.1. The effect of extraction time on PRG yield

One of the important factors to determine the PRG extraction efficiency was the extraction time. The PRG yield increased as the extraction time, while the yield of PRG almost kept unchanged after 4 h, and the yield was 4.15%. Thus, 4 h was chosen as the center point of extraction time.

3.1.2. The effect of temperature on PRG yield

Higher extraction temperature can sufficient break the cell wall structure and release the PRG from the cells. It is widely acknowledged that the temperature is such an important factor in the polysaccharide extraction process. For obtaining the optimal temperature on the yield of PRG, we extracted the PRG and calculated the yield at various temperatures (50, 60, 70, 80 and 90 °C) with the extraction time 3 h and liquid-solid ratio 40 g/L. In Fig. 1b, the PRG yield increased ranging from 50 to 90 °C, and the maximum yield of PRG was 4.69% at 90 °C. According to the results

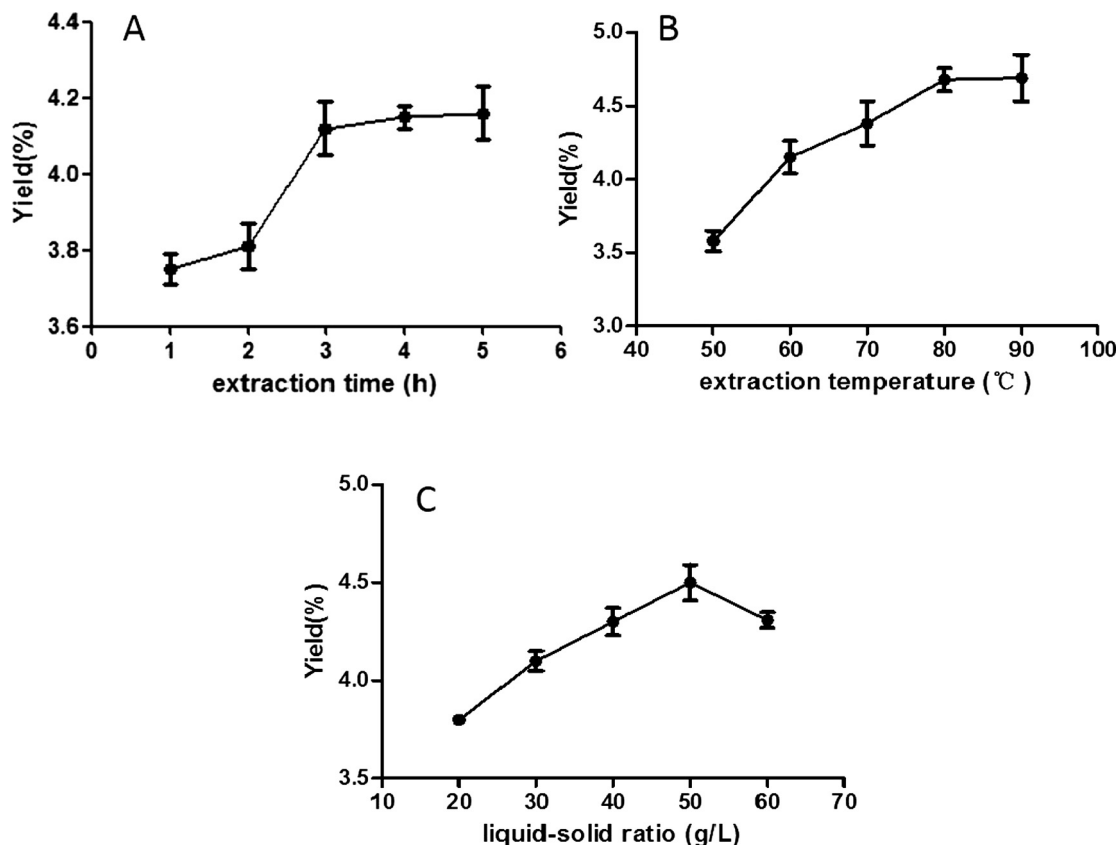


Fig. 1. The effects of extraction conditions on the PRG yield. (A) Extraction time. (B) Extraction temperature. (C) Liquid-solid ratio.

above, the extraction temperature range of 70–90 °C was used for further BBD experiment (see Fig. 2).

3.1.3. The effect of liquid-solid ratio on PRG yield

To confirm the optimal liquid-solid ratio to extract PRG, different of liquid-solid ratio were set at 20, 30, 40, 50 and 60 g/L, respectively. In Fig. 1c, the yield of PRG increased from 20 to 50 g/L, while, PRG yield decreased when the liquid-solid ratio to material was at 60 g/L. For the materials, lower liquid-solid ratio can completely enhance the driving force transfer of the materials to water, however, higher liquid-solid ratio might contribute to the lower yield of PRG. Hence, 40 g/L was used as the center point in further experiment.

3.2. Extraction optimization by RSM

3.2.1. Predicted model and statistical analysis

Based on the results of single-factor design above, a 17-run BBD experiments of three factors and three levels at the center point were applied to confirm the optimum extraction levels for the three factors including the extraction temperature, time and liquid-solid ratio. Our results of PRG yield under various conditions are displayed in Table 2, and the results was analyzed by design expert software. The second-order polynomial equation was used to explore the relationship between PRG yield and the designed factors, which was calculated as below:

$$Y = 0.20X_1 + 0.26X_2 + 0.12X_3 - 0.52X_1X_2 - 0.348X_2X_3 + 0.13X_1X_3 - 0.19X_1^2 - 0.63X_2^2 - 0.69X_3^2 + 5.36$$

where Y denotes the extraction yield of PRG, X_1 , X_2 and X_3 are the extraction time, extraction temperature and liquid-solid ratio respectively.

In the results of BBD, the *F*- and *P* values were considered as tools to determine the significance of each coefficient. The greater the *F*-values and the smaller the *P* values were, the more significant the corresponding coefficient. ANOVA was applied to evaluate the predicative factors and model. The *F* value was 32.96 and *P* value was lower than 0.0001 in Table 3, which suggested that the model was significant. The determination coefficient R^2 of this model was 0.98 which indicated that 98% of the variability in the response values could be explained. The adjusted determination coefficient value ($R^2 = 0.95$) also suggested that this model was highly significant. Moreover, the *P*-value of 0.2849 for lack-of-fit implied the lack-of-fit was not significant relative to pure error, which also indicated the model equation was adequate to predict the PRG yield within the range of experimental variables.

3.2.2. Optimization and validation for extraction of PRG

Both the three-dimensional of response surface and two-dimensional con-tour plots were applied to evaluate the interactions of the designed factors and yields of PRG. The results of the contour plots suggested if the mutual interactions between factors are significant or not. The response surface plots and contour plots depicted the mutual interactions between the factors by keeping the other factors at their zero levels for PRG yield (Fig. 1). Within the three factors, the interactions between extraction temperature and time in Fig. 1A, temperature and liquid-solid ratio in Fig. 1C were significant, but the interaction between extraction time and liquid-solid ratio in Fig. 1C was not significant. Through the Design Expert software, the solved optimal levels of the designed factors for the PRG extraction were extraction time 4 h, extraction temperature 77.3 °C, liquid-solid ratio 42.5 g/L. To confirm the adequacy of model equation, 5 additional experiments under the optimum

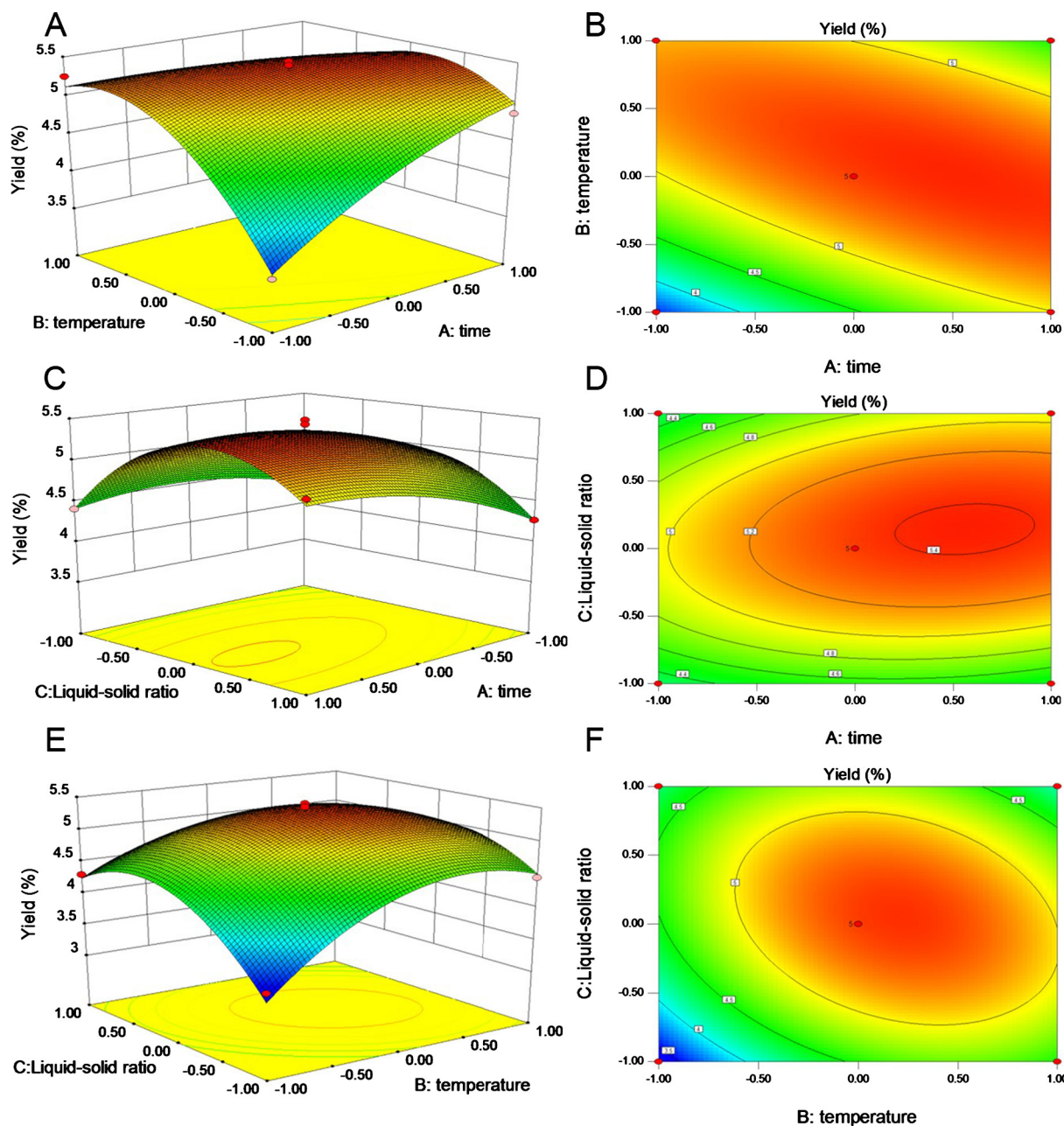


Fig. 2. The response surface plot (A, C and E) and contour plots (B, D and F) indicating the effects of factors (X_1 , extraction time, X_2 , extraction temperature, X_3 , liquid-solid ratio) and their interactions on the PRG yield.

extraction conditions were carried out. The PRG yield was $5.42 \pm 0.12\%$, which is very closed with the predicted value of 5.43% . Our results suggested the regression model was accurate and adequate for the prediction of PRG extraction.

3.3. Measurement of antioxidant activity of PRG

3.3.1. DPPH free radical scavenging activity assay

As reported, tDPPH radical is recognized as a stable free radical which has been widely considered as a tool for measurement of the free radical scavenging activities of antioxidants, such as the extracts from plants and microorganism, natural compounds and synthetic pure compounds (Li et al., 2008). PRG showed dose-dependent scavenging activity from 1 to 6 mg/mL against the DPPH radical in Fig. 3A. As the concentration increased to 5.0 mg/

mL, the scavenging activity was almost stable. At 6.0 mg/mL, the most effective DPPH scavenging activity was observed, and it was 75%.

3.3.2. ABTS free radical scavenging activity

PRG exhibited a concentration-dependent manner ranging (Fig. 3B). At the dose of 6 mg/mL, our results showed that PRG has the strongest ABTS free radical scavenging activity, the value was 71%.

3.3.3. Hydroxyl radical scavenging activity

The hydroxyl radical is considered to be the most reactive in the reactive oxygen species, which could induce damage to adjacent biomolecules, and cause several diseases (Jeong et al., 2009). In Fig. 3C, As the PRG concentration increased from 1 to 6 mg/mL,

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

Source	Sum of squares	DF	Mean square	F-value	P-value
Model	6.77	9	0.75	32.96	<0.0001 ^c
X ₁	0.31	1	0.31	13.49	0.0079 ^b
X ₂	0.55	1	0.55	23.91	0.0018 ^b
X ₃	0.12	1	0.12	5.47	0.0519 ^d
X ₁ X ₂	1.06	1	1.06	46.45	0.0002 ^c
X ₁ X ₃	0.070	1	0.070	3.07	0.1230 ^d
X ₂ X ₃	0.47	1	0.47	20.54	0.0027 ^b
X ₁ ²	0.16	1	0.16	6.83	0.0347 ^a
X ₂ ²	1.66	1	1.66	72.59	<0.0001 ^c
X ₃ ²	2.03	1	2.03	89.05	<0.0001 ^c
Residual	0.16	7	0.023		
Lack of fit	0.092	3	0.031	1.81	0.2849 ^d
Pure error	0.068	4	0.017		
Cor total	6.93	16			

R² = 0.98, adjusted R² = 0.95, C.V.% = 3.25%.

^a Significant, p < 0.05.

^b Very significant, p < 0.01.

^c Highly significant, p < 0.001.

^d Non-significant, p > 0.05.

the hydroxyl radical scavenging activity became stronger. The results showed that PRG clearly displayed scavenging activity against hydroxyl radical scavenging activity.

3.3.4. Superoxide radical assay

For the intent of better understanding the PRG capacity to inhibit peroxidation, and assay was conduct to measure the scavenging of superoxide radical. PRG exhibited a strong activity against superoxide radical. In Fig. 3D, The PRG showed dose-dependent scavenging activity. Comparison with DPPH and ABTS, PRG showed higher superoxide radical scavenging activity. The maximum of scavenging activity was observed at 6.0 mg/mL, and it was 75%.

3.4. Toxicity of PRG against Hela and Siha cells

A dose-dependent cell viability reduction in Hela and Siha cells were observed after PRG incubation. The 24-h IC₅₀ of PRG in Hela and Siha were approximately 156 µg/mL and 186 µg/mL, respectively (Fig. 4). The data was associated with previously reports which revealed many natural polysaccharides possessed potent anti-tumor activities (Xu et al., 2016; Tian et al., 2016).

4. Discussion

Polysaccharide from mushroom, possess wide-ranging beneficial therapeutic effects and health-promoting properties including

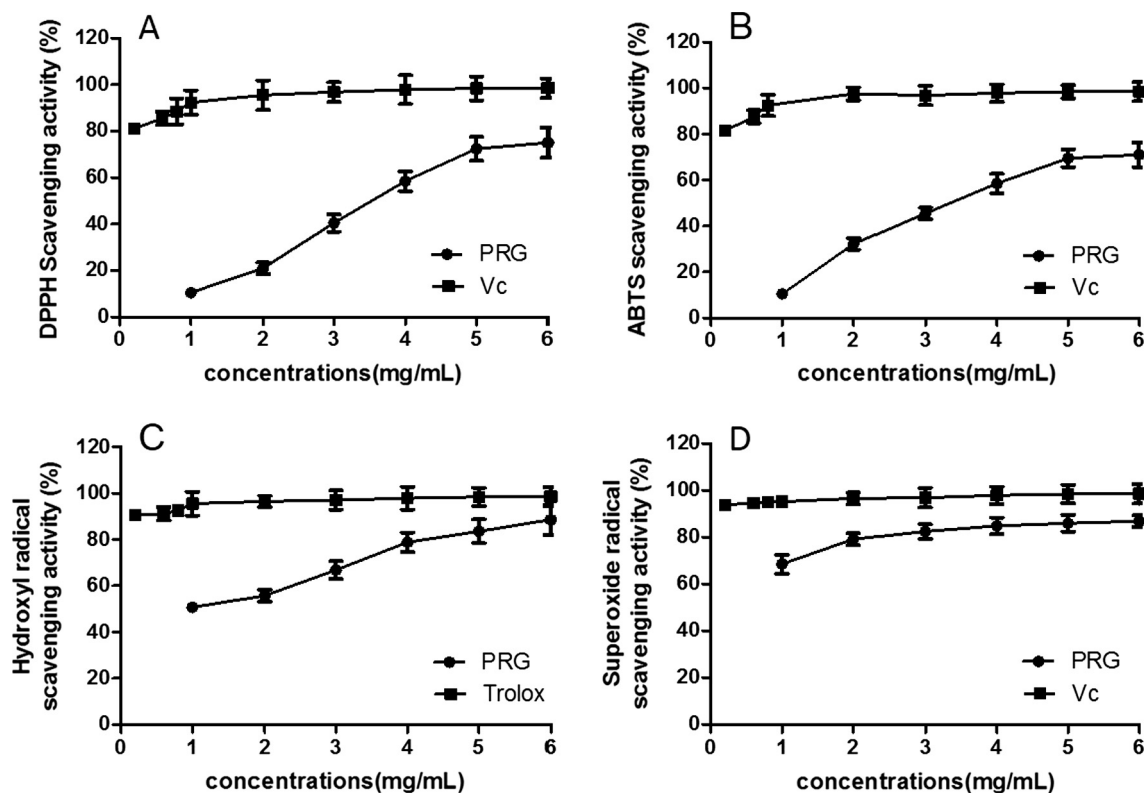


Fig. 3. Antioxidant activities of PRG. (A) DPPH radical scavenging activity, (B) ABTS radical scavenging activity, (C) hydroxyl radical scavenging activity, (D) superoxide radical scavenging activity.

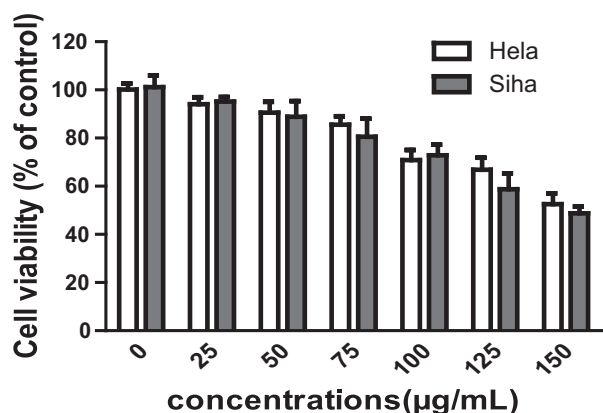


Fig. 4. PRG induced cell damage in both HeLa and SiHa cells. PRG dose-dependently suppressed cell viability after 24 h.

anti-tumor, inflammation modulation and anti-oxidant activity, has attracted considerable attention recently. (Feng et al., 2016; Ji et al., 2016; Roy et al., 2016; Zhang et al., 2016; Palanisamy et al., 2016). However, the biological activities of wild *R. griseocarnosa* have not been reported systematically yet.

Extraction technique is closely relative with polysaccharide biological activities. Hence, the parameters of polysaccharide extraction were firstly optimized in this study. The optimal parameter was predicted by RSM method. The objective of RSM is to determine the levels of experimental variables which allow obtaining a maximum extract yield of PGF from *R. griseocarnosa* sporocarp. As reported, RSM was widely used in the polysaccharides extraction. The optimal extraction conditions of *Artemisia sphaerocephala* Krasch seeds polysaccharide, and polysaccharide with antioxidant activity from Tunisian *Zizyphus lotus* fruit were obtained using RSM (Xie et al., 2016; Zheng et al., 2016; Khaoula et al., 2016).

The preliminary ranges of the three key factors were determined by single factor design. The temperature is such an important variable in the polysaccharide extraction process. Since higher temperature can destroy effectively the cell wall structure and release polysaccharides diffusion from cells. As reported, the best extraction temperature of a major polysaccharide with potential antioxidant and anti-tumor activity in human colon cancer from *Sargassum thunbergii* was as high as 97.29 °C (Yuan et al., 2015; Sarfraz et al., 2017). In this study, 70–90 °C were chosen to be the preliminary range on the basis of results (Fig. 1B). As for liquid-solid ratio, PRG yield increased from 20 to 50 g/L, while, PRG decreased when the liquid-solid ratio was at 60 g/L (Fig. 1C). As the literature reported, if liquid-solid ratio was too low, polysaccharide dry power could not be completely extracted up (Zhu and Liu, 2013), in contrast, it would cause high cost of material. Therefore liquid-ratio range was chosen as 30–50 g/L. Additionally, the extraction time is another important variable which would influence the extraction yield. With the extraction time increased to 3 h, PRG yield was significantly higher than before (Fig. 1A). PRG yield approached a maximum at 5 h. After 5 h, the yield of PRG maintained a dynamic equilibrium. This phenomenon is probably because PRG has been already sufficiently extracted up in 5 h.

Based on the single factor experiment, 17 run trials were applied to the RSM according to BBD. The model was valued, the determination coefficient R^2 was 0.98, and the adjusted determination coefficient was 0.95. The results indicated this model could be used for the prediction of PRG yield. The average yield of PRG was $5.42 \pm 0.12\%$ using the optimized parameters, which is closed to the predicted yield.

Excessive free radicals and reactive oxygen species are able to induce damage to DNA, RNA, membrane lipids, protein and other

substances (Ji et al., 2016; Roy et al., 2016; Sun et al., 2016). A soluble β -glucan purified from the alkaline extract of ectomycorrhizal edible mushroom *Russula albonigra* (Krombh.) Fr. Exhibited potent antioxidant activities (Nandi et al., 2014). Hence, the antioxidant activity of crude polysaccharide from *R. griseocarnosa* sporocarp was investigated. In this study, the crude polysaccharide of *R. griseocarnosa* sporocarp displayed higher ability of scavenging of DPPH, ABTS, hydroxyl radicals and superoxide radical. DPPH free radical assay has been applied to evaluate the free radical scavenging ability of various compound (Safi et al., 2015; Tan and Lim, 2015). ABTS assay was also widely employed as an index to determine the antioxidant activity of test samples (Benso et al., 2016). Hydroxyl radicals could almost react with all the biomacromolecules in living cells and cause damage to the adjacent bio-molecules (Cheng et al., 2002). Though superoxide radical was a weak oxidant, it can induce damage to the DNA and cells leading to various diseases (Yine et al., 2015; Chiste et al., 2015; Rahman et al., 2017). Our results suggested that *R. griseocarnosa* polysaccharide exhibited antioxidant activities including DPPH, ABTS, hydroxyl radicals and superoxide radical. PRG is potent to be source of natural antioxidants. Although the antioxidant activity of PRG has been studied, further work is needed to elucidate the mechanism. The anti-tumor activity was investigated. PRG exhibited capability of anti-proliferation in HeLa and SiHa cells. Though the anti-tumor activity of PRG was lower than synthetic agents such as cis-platin, PRG like other polysaccharides has the advantage of lower cytotoxicity. It is worth to explore the natural polysaccharides as potential anti-tumor agents, although their anti-tumor concentrations were usually presented in high levels.

5. Conclusion

In this study, RSM was applied to obtain crude polysaccharides from wild *R. griseocarnosa*. The optimal extraction parameter analyzed by BBD is as follows: extraction temperature 77.3 °C, extracting time 4 h, and the ratio of water to material 42.5 g/L. Under this optimal condition, the experimental yield of PRG was $5.42 \pm 0.12\%$, which is in good agreement with predicted value 5.43%.

PRG displayed stronger DPPH, ABTS, Hydroxyl radical and superoxide radical scavenging activities. PRG also displayed the significant anti-proliferative effect on HeLa and SiHa cells. The results may offer new insights into the development and utilization of polysaccharide from *R. griseocarnosa*.

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