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Extraction optimization of *Bruguiera gymnorrhiza* polysaccharides with radical scavenging activities

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ABSTRACT

Box–Behnken design including independent variables such as extraction temperature (60–80 °C), extraction time (20–40 min) and ratio of water to raw material (30–50 mL/g) was used to optimize the extraction process of *Bruguiera gymnorrhiza* polysaccharides (BGPs). The experimental data were fitted to a second-order polynomial equation using multiple regression analysis. The optimum conditions were predicted as follows: extraction temperature 71 °C, extracting time 31.4 min, and ratio of water to raw material 42. Under these conditions, the yield of BGPs obtained was (16.43 ± 0.08)%, which was in good agreement with the predicted value 16.47%. Additionally, characterization of BGPs was obtained by FT-IR analysis. The antioxidant activities of BGPs were evaluated *in vitro*. BGPs demonstrated appreciable antioxidant potential on superoxide anion radical, ABTS radical, and hydroxyl radical scavenging. These may provide theoretical basis for further system research and rational development and utilization of mangrove resources.

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1. Introduction

Bruguiera gymnorrhiza, which belongs to the family Rhizophoraceae, is reported to be one of the dominant tree species in Chinese mangrove, and widely distributed on seashores and edges throughout Guangxi, Guangdong, Hainan, Fujian and Zhejiang provinces of China. It is also naturally distributed in Southeast Asia, southern and eastern Africa, Australia, Micronesia and Polynesia (Hou, 1970; Duke, 1992; Lin, 1999). Up to date, several researches have demonstrated that not only *B. gymnorrhiza* is used as a wood product, but also it has various biological functions and has even been suggested as anti-febrile, anti-diarrhea, anti-inflammatory and bactericidal activities as early as in folk pharmacology in China (Lin, Lin, Yang, & Wang, 2005; Yu, Jin, & Ye, 2009). Traditionally, the fruits of *B. gymnorrhiza* were prescribed to treat diarrhea, and the roots and leaves were used to treat burns (Othman, 1998; Bamroongrugsa, 1999). Additionally, its flowers were thought to be a vegetable in Thailand (Bunyapraphatsara, 2000). Previous studies showed that extracts and chemicals from Mangrove species were used mainly in folkloric medicine as insecticides and pesticides (Bandaranayake, 2002). According to the present studies, ethanol or methanol extract of *B. gymnorrhiza* trunks showed antifungal activity and that of *B. gymnorrhiza* barks could inhibit growth of all pathogenic bacteria (Kazuhiko, 2002; Haq, Sani, Hossain, Taha, & Monneruzzaman, 2011). The leaves of *B. gymnorrhiza* have long attracted attentions as traditional medicines for their a variety of bioactive substances, such as fatty acids, amino acids, flavonoids and terpenes (Shang & Long, 2005; Zhang & Lin, 2008; Zheng et al., 2008; Fan, Song, Lin, Gu, & Xu, 2009). However, to our knowledge, little attention was devoted to the extraction of *B. gymnorrhiza* polysaccharides (BGPs), especially detailed studies on extraction procedure and their bioactivity. Therefore, we reported the optimization of extracting parameters for the production and its preliminary characterization of BGPs.

Existing studies have observed that many polysaccharides from natural sources are widely employed in industry due to

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their biocompatibility, biodegradation, non-toxicity and non-immunogenicity (Gao, Wang, Su, Zhang, & Yang, 2012; Luo, 2012; Ye & Huang, 2012; Yu, Wang, Wei, & Dong, 2012; Yu & Chao, 2013). It is worth noting that most antioxidants are currently synthesized industrially and are suspected of being responsible for liver damage and carcinogenesis. Therefore, it is essential to develop and utilize effective and natural antioxidant to protect the body from reactive oxygen species (ROS) damage. Consequently, various naturally occurring substances are receiving continuous attention as antioxidants. Published data indicate that polysaccharides from plants have certain antioxidant activity on free radicals and can be explored as novel potential antioxidants (Capek, Machová, & Turjan, 2009; Luo et al., 2010; Gan & Latiff, 2011; Han, Jiang, & Zhang, 2011; Chen, Zhang, Jiang, Mu, & Miao, 2012).

The present study was conducted to optimize extracting parameters using response surface methodology (RSM) to study the effects of extraction conditions on the extraction yield of BGPs. Besides, *in vitro* antioxidant activities of BGPs, such as superoxide, ABTS radicals and hydroxyl radicals, were evaluated.

2. Materials and methods

2.1. Materials and chemicals

Dried leaves of *B. gymnorrhiza* were purchased from Tianjin, China. Ferrous sulfate ($FeSO_4$), ferrozine, nitroblue tetrazolium (NBT), 2-2-azino-bis-(3-ethyl-benzthia-zoline-6-sulfonic acid) (ABTS), and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Extraction of BGPs

The dried leaves of *B. gymnorrhiza* were ground in a rotary mill and then sieved (40 mesh) to obtain a fine powder as pretreated samples. In order to defat and remove some colored materials, oligosaccharides, and some small molecule materials, each pretreated sample (100 g) were added into 80% ethanol (100 mL) with 70 °C water bath for 2 h, according to Li, Ding, and Ding (2007) and Luo et al. (2011) with some modifications. After dried at room temperature, each sample was extracted by deionized water in a designed extraction temperature, extraction time, and ratio of water to raw material. Each extract was left to cool at room temperature, filtered, and then the resulting solutions were precipitated by the addition of ethanol to a final concentration of 80% (v/v) and kept overnight at 4 °C. The precipitates collected by centrifugation (4000 rpm, 5 min) were washed three times with absolute ethanol and dried under reduced pressure. The crude extract named BGPs obtained was weighted with a balance (BS2202S, SARTORIUS, Germany). And the yield (%) of BGPs in the extraction was calculated by the following:

$$\text{Extraction yield (\%, w/w)} = \frac{\text{weight of dried crude polysaccharide extraction (g)}}{\text{weight of powders (g)}} \times 100 \quad (1)$$

2.3. Experimental design

Effects of extraction conditions such as extraction temperature, extraction time, ratio of water to raw material, extraction number, ethanol concentration and particle size, on extraction yield of BGPs, have been investigated by the single factor method. By the single factor experimental results, three major influence factors (extraction temperature, extraction time, ratio of water to raw material) were confirmed. As for the preliminary range of the three factors for the next design experiment, the effect of extraction temperature, extraction time and ratio of water to raw material on the extraction yield of BGPs was studied by single-factor design. In detail, each pretreated sample mentioned above was extracted under extraction temperature (50–90 °C), extraction time (10–50 min) and ratio of water to raw material (10–50 mL/g). One factor is changed, while the other factors keep constant in each experiment. The effect of each factor was evaluated by determining the extraction yield of BGPs. On the basis of the single-factor experimental results, three major influence factors and the ranges of each factor were confirmed. Then, a three-variable, three-level Box–Behnken design (BBD) (Prakash Maran, Manikandan, Thirugnanasambandham, Vigna Nivetha, & Dinesh, 2013; Prakash Maran, Sivakumar, Sridhar, & Thirugnanasambandham, 2013; Santos, Espeleta, Branco, & de Assis, 2013) was employed in this optimization study based on the results of preliminary single-factor-test, as shown in Table 1. Three independent variables (X_1, extraction temperature; X_2, extraction time; X_3, ratio of water to raw material) at three levels were performed.

Table 1
Levels and code of variable used for Box–Behnken design (BBD).

Independent variables	Symbol	Range and level		
		−1	0	1
Extraction temperature (°C)	X_1	60	70	80
Extraction time (min)	X_2	20	30	40
Ratio of water to raw material (mL/g)	X_3	30	40	50

The coded values of the extraction parameters were determined by the following equation:

$$X_i = \frac{x_i - x_0}{\Delta x}, \quad i = 1, 2, 3, \quad (2)$$

where X_i represents a coded value of the variable; x_i represents the actual value; x_0 represents the actual value of the independent variable at the center point; and Δx represents the step change of variable.

A design matrix comprising of 17 experimental runs was constructed, as shown in Table 2. The non-linear computer-generated quadratic model used in the response surface is given as

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=0}^{3}\beta_{ii} X_i^2 + \sum_{i=0}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j, \quad (3)$$

Table 2
BBD with the observed responses for the extraction yield of BGPs.

Run	Coded variable levels			Extraction yield (%)
	X_1 (temperature)	X_2 (time)	X_3 (ratio)	Experimental values
1	−1	−1	0	12.85
2	1	−1	0	16.30
3	−1	1	0	15.87
4	1	1	0	14.21
5	−1	0	−1	12.70
6	1	0	−1	15.82
7	−1	0	1	13.21
8	1	0	1	12.98
9	0	−1	−1	13.75
10	0	1	−1	16.87
11	0	−1	1	13.70
12	0	1	1	12.24
13	0	0	0	17.02
14	0	0	0	14.26
15	0	0	0	13.54
16	0	0	0	14.10
17	0	0	0	14.52

where β_0, β_i, β_{ii}, and β_{ij}, represent the regression coefficients for intercept, linear, quadratic and interaction terms, respectively; X_i and X_j represent the coded independent variables; and Y represents the predicted response. The response surface graphs of predicted values by models were plotted using the Design-Expert software (version 8.0, Stat-Ease, Inc, Minneapolis, USA). The analysis of variance tables was generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The values of R^2, adjusted-R^2 of models were evaluated to check the model adequacies. And additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design. P-values of less than 0.05 were considered to be statistically significant.

2.4. FT-IR spectroscopy

One milligram of BGPs was milled with 300 mg of KBr, and pressed into a disk for transmission infrared spectroscopy (Guo, Zou, & Sun, 2010). Then, Fourier Transform Infrared (FT-IR) spectra were determined at a phase resolution of 4 cm^{-1} and averaging 30 scans/min, using a STIR-8400S IR spectrometer (USA) in the frequency range of 4000–400 cm^{-1} (Yamamoto, Nunome, Yamauchi, Kato, & Sone, 1995). Three replicate spectra were obtained.

2.5. Superoxide anion radical scavenging activity

The superoxide radical scavenging abilities were assessed based on the capacity of the sample to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the NADH–NBT–PMS system (Naguib, 2000; Kanatt, Chander, & Sharma, 2007). In the present study, sample solution was treated with 4.5 mL of 16 mM Tris–HCl buffer (pH 8.0) containing 300 μM NBT (0.5 mL), 468 μM NADH (0.5 mL) and 3.5 mL sample solution at different concentration (0.5–50.0 μg/mL). The reaction was initiated by adding 0.5 mL of PMS (60 μM in 20 mM potassium phosphate buffer pH 7.4) to the mixture. The mixture was incubated at room temperature for 5 min and the absorbance was measured at 560 nm against a blank sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity. In the assay, BHT was used as a positive control, and a control contained all the reaction reagents except the samples or positive control was prepared. The scavenging effect on the superoxide anion radicals was calculated using the following equation:

$$\text{Scavenging effect}\ (\%) = \frac{A_{\text{control}\,560} - A_{\text{sample}\,560}}{A_{\text{control}\,560}} \times 100, \quad (4)$$

where $A_{\text{sample560}}$ represents the absorbance of sample group and $A_{\text{control560}}$ represents the absorbance of blank control group. All the absorbencies were read under 560 nm.

2.6. ABTS radicals

The radical scavenging activity of BGPs against ABTS was measured using the method with some modifications (Katalinic, Milos, Kulisic, & Jukic, 2006). The ABTS radical cation ($ABTS^+$) was generated by mixing an ABTS (7 mM) solution with a potassium persulfate (2.45 mM) aqueous solution and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C for 30 min. Then 2.0 mL of the $ABTS^+$ solution was added to 0.2 mL of various concentrations (0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL) of the BGP solutions. After reacting for 20 min at room temperature, the absorbance was measured at 734 nm. BHT was used as standard. The scavenging activity of ABTS (%) was calculated according to the following equation:

$$\text{ABTS scavenging effect}\ (\%) = \frac{A_{\text{blank}\,734} - A_{\text{sample}\,734}}{A_{\text{blank}\,734}} \times 100, \quad (5)$$

where A_{blank734} represents the absorbance of ABTS without sample and $A_{\text{sample734}}$ represents the absorbance of sample and ABTS. All the absorbencies were read under 734 nm.

2.7. Hydroxyl radicals

The hydroxyl radical-scavenging ability of polysaccharides was measured according to Fenton method described before (Halliwell, Gutteridge, & Aruoma, 1987; Zhong, Jin, Lai, Lin, & Jiang, 2010), with minor modifications. Samples were dissolved in distilled water to form final concentrations of 0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL and incubated with 9.0 mM $FeSO_4$ (1.0 mL), 0.3% H_2O_2 (1.0 mL) in 0.5 mL salicylic acid–ethanol solution (9.0 mM) for 30 min at 37 °C. The absorbance of the mixture was read at 510 nm against a blank. The total volume of the mixture in each tube was made up to 3 mL by adding the required amount of distilled water. Scavenging activity of hydroxyl radical calculated as follows:

$$\text{Hydroxyl radical scavenging effect}\ (\%) = \frac{A_{\text{blank}\,510} - A_{\text{sample}\,510}}{A_{\text{blank}\,510}} \times 100, \quad (6)$$

where A_{blank510} and $A_{\text{sample510}}$ represent the absorbance of blank control group and sample group under 510 nm.

2.8. Statistical analyses

Statistical analysis was performed using Design-Expert (Version 8.0, Stat-Ease) and SPSS (Version 17.0) statistical software. Data were analyzed using Student's t-test and F-test, and P values of <0.05 were considered statistically significant. For multiple comparisons, one-way analysis of variance (ANOVA) was used.

3. Results and discussion

3.1. Optimization of BGP extraction process

3.1.1. Single-factor experiments

In this part, extraction temperature, extraction time and ratio of water to raw material were studied, respectively, when other extraction conditions were described in Section 2.2, and the results of single factor experiments are presented in Fig. 1. As can be seen from Fig. 1, the three factors were found to have a significant impact on the yield of BGPs.

As shown in Fig. 1(A), the effect of temperature on extraction yield of BGPs was investigated. To study the effect of different temperatures on the yield of BGPs, the extraction process was carried out at 50, 60, 70, 80 and 90 °C, while the other two extraction variables were set as follows: extraction time 30 min and ratio of water to raw material 40 mL/g. Fig. 1(A) showed that the extraction yield increased up to its maximum amount at 70 °C, and the maximal yield (15.50 ± 1.57)% of BGPs was obtained. Then, the yield of BGPs no longer increased when extraction temperature continued to rise. This tendency is in good agreement with some researches of other authors (Guo et al., 2010; Yang et al., 2010; Yu & Chao, 2013). This result indicated that temperature enhanced the BGP extraction from the *B. gymnorrhiza* leave particles into the water to a certain level, followed by their possible loss due to decomposition at a higher temperature.

The extraction time was another factor that would influence the extraction yield. It has been reported that a longer extraction

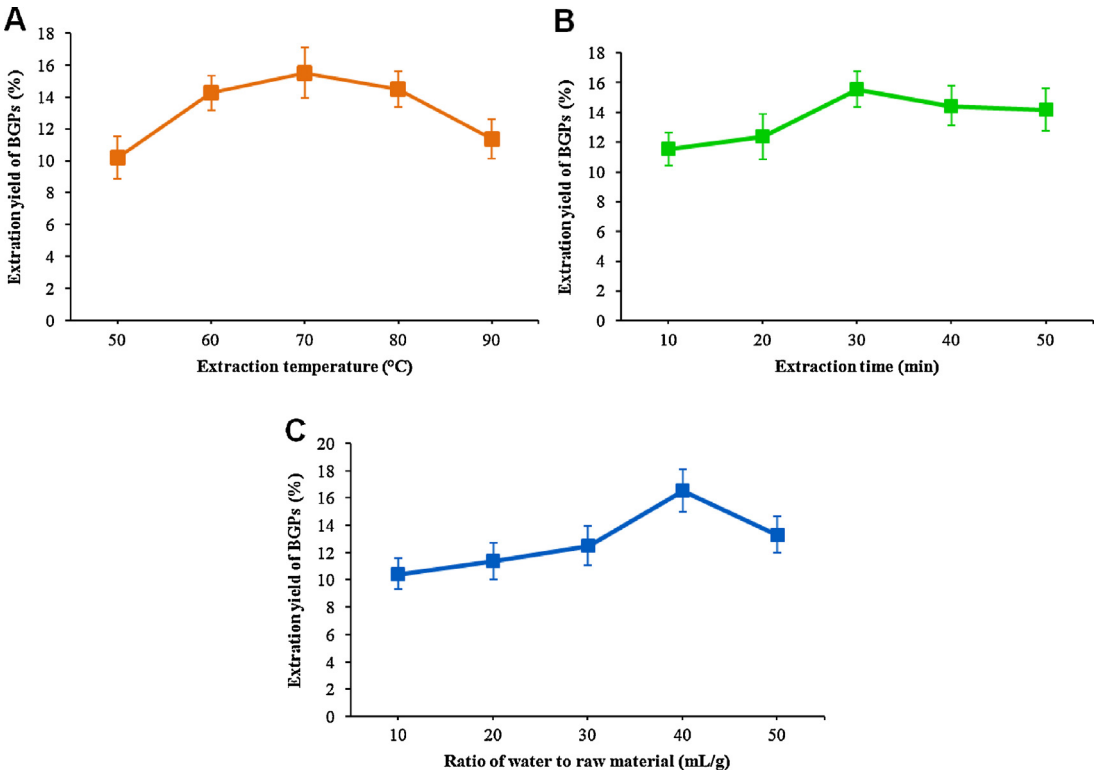

Fig. 1. Effect of extraction temperature (A), extraction time (B) and ratio of water to raw material (C) on the extraction yield of BGPs.

time favors the production of polysaccharides (Ros et al., 2004; Liu, Wei, Guo, & Kennedy, 2006; Yin & Dang, 2008; Yu & Chao, 2013). As shown in Fig. 1(B), the yield of the BGPs increased with the increase of extraction time. When the extraction time increased from 10 to 50 min, other experimental conditions were as follows: extraction temperature 70 °C and ratio of water to raw material 40 mL/g. The extraction yield of BGPs reached a maximal value of (15.57 ± 1.21)% at 30 min, and no longer changed as the extraction time prolonged, as seen in Fig. 1(B). Therefore, time range 20–40 min was selected as the optimal in the BBD experiment with saving cost taken into consideration.

As an important extraction parameter, ratio of water to raw material will significantly affect extraction yield (Govender et al., 2005). This effect on the yield of BGPs is shown in Fig. 1(C). In the present studies, ratio of water to raw material was set at 10, 20, 30, 40 and 50 mL/g, when the extraction temperature and extraction time was fixed at 70 °C and 30 min. The extraction yield of BGPs increased with increasing ratio of water to raw material, and the highest value (16.52 ± 1.56) % was obtained with the ratio increasing from 10 to 40. This is probably due to the increase in the driving force for the mass transfer of polysaccharides (Bendahou, Dufresne, Kaddami, & Habibi, 2007). However, the results from the present experiments indicated that when the ratio is higher than 40, the polysaccharides yield was decreased. This might be due to the reason mentioned by Yin and Dang (2008).

Hence, the preliminary ranges (extraction temperature 60–80 °C; extraction time 20–40 min; ratio of water to raw material 30–50 mL/g) of the three factors were chosen for further optimization in BBD design.

3.1.2. Optimization of BGP extraction progress in BBD assay

The extraction conditions certainly affected the extraction yield of BGPs. The experiments were designed to evaluate the effects of the three independent variables including extraction temperature (X_1), extraction time (X_2), and ratio of water to raw material (X_3) on the extraction yield (%) of BGPs, as shown in Table 2. Results also showed that the yield of polysaccharides ranged from 12.24% to 17.02%. The response was sufficiently explained by the predicted model, and the response variable and the test variables are related by the following second-order polynomial equation:

$$EY = 16.38 + 0.21X_1 + 0.44X_2 + 0.54X_3 + 0.41X_1X_2 - 0.24X_1X_3 - 0.17X_2X_3 - 1.48X_1^2 - 1.57X_2^2 - 1.26X_3^2 \quad (7)$$

The predicted values of EY (%) of BGPs were calculated using the regression model and compared with experimental values in Fig. 2, which shows that the actual response values agree well with the predicted response values.

The analysis of variance (ANOVA) for the model is given in Table 3. The p-values were used as a tool to check the significance of each coefficient, and the smaller the p-value was, the more significant the corresponding coefficient was (Guo et al., 2010). According to this model, the quadratic term of extraction temperature ($X_1{}^2$), extraction time ($X_2{}^2$) and ratio of water to raw material ($X_3{}^2$) indicated that the variables had the largest effect ($p < 0.001$). Results also showed that the linear terms of extraction time (X_2) and ratio of water to raw material (X_3) were the significant impact factors on the extraction yield of BGPs due to it having the p-value which was less than 0.05, while that of the other linear term extraction temperature (X_1) was more than 0.05. The "Lack of Fit" ($p > 0.05$) implied the Lack of Fit was not significant relative to the pure error.

After the response surface regression procedure, the total determination coefficient (R^2) was 0.9609, indicating a reasonable fit of the model to the experimental data, as shown in Table 3. The value of the adjusted determination coefficient (Adj $R^2 = 0.9107$) also confirmed that the model was highly significant. Moreover, a low value of coefficient of the variation (C.V.) (3.12%) clearly indicated a high degree of precision and a good deal of reliability for the experimental values. Besides, the "Adeq precision" of 11.570 indicated that this model could be used to navigate the design space.

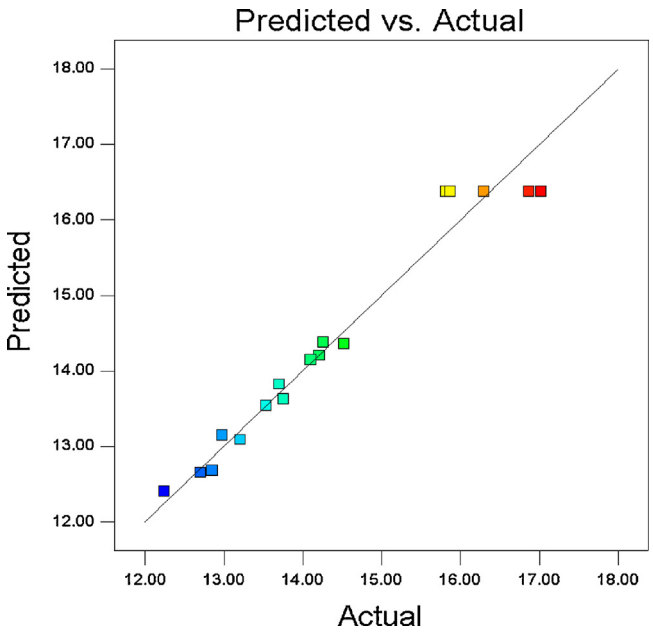

Fig. 2. Predicted response *versus* actual value.

The aim of this present study was to find the optimum conditions which give the maximum extraction yield of BGPs. The relationship between independent and dependent variables is illustrated by the 3D response surfaces and the 2D contour plots generated by the model using the Design-Expert software. The shapes of the contour plots indicate whether the mutual interactions between the variables are significant or not. Two variables were depicted in a 3D response surface and a 2D contour plot while the other variable kept at level zero, as shown in Fig. 3. The interaction relationships of extraction temperature (X_1), extraction time (X_2), and ratio of water to raw material (X_3) on the extraction yield (*EY*) of BGPs are shown in Fig. 3. An elliptical contour plot means the interactions between the variables are significant while a circular contour plot means not (Yan et al., 2011). As shown in Fig. 3, the *EY* of BGPs are not affected significantly by alterations of the variables through three independent response surface plots and their respective contour plots in the experimental range. All the three variables X_1, X_2 and X_3 have a positive impact on *EY*. But it can be seen from Fig. 3A and A′ that *EY* significantly increased upon increasing the extraction temperature to approximately 70 °C, but decreased sharply beyond this duration, reaching a maximum *EY* at 16.2%. The effect of the extraction time on *EY* of BGPs was additionally sensitive within the tested range, reaching a maximum *EY* at approximately 16.2%. The same trends were depicted in other figures of Fig. 3.

By analyzing the plots, the optimal conditions for BGP extraction obtained were extraction temperature at 71 °C, extracting time for 31.4 min, and ratio of water to raw material at 42. In the optimal conditions, the maximum predicted yield of BGPs was 16.47%, and the experiment yield of BGPs was (16.43 ± 0.08)%, which agreed with the predicted value. Therefore, the results indicated suitability of the model employed and the success of RSM in optimizing the extraction conditions for BGPs.

3.2. FT-IR analysis

Among the complex polysaccharide sources, the identification of polysaccharides could be a key factor if a rapid and reliable method is attainable. Wet classical chemical methods of polysaccharide determination are time consuming, and not always straight forward, hence not suitable for a widespread routine application in the drug industry. Among present methods, FT-IR spectra have been employed for their identification and discrimination (Chopin & Whalen, 1993; Sekkal & Legrand, 1993). To have a precise characterization of the BGP, the FT-IR spectra of BGP were recorded at the range of 4000–400 cm^{-1} (Fig. 4). Typical signals of BGP at 3440.7, 2921.9, 2358.8, 1731.9, 1643.2, 1417.6 and 1070.4 cm^{-1} were clear for the sample. It displayed a broad and intense peak around 3410 cm^{-1}, which were due to the hydroxyl groups stretching vibration. And the peak at 2921.9 cm^{-1} was assigned to C—H stretch vibration. The absorption 2358.8 cm^{-1} indicated the existence of aliphatic C—H bond. The peak 1731.9 cm^{-1} and 1643.2 cm^{-1} represented the ester carbonyl groups (C═O) and carboxylate (COO—)

Table 3
ANOVA for response surface quadratic model.

Variables	Sum of squares	*DF*	Mean square	*F* value	*P*-value Prob. > *F*
Model	34.51	9	3.83	19.13	0.0004[a]
X_1	0.34	1	0.34	1.70	0.2337
X_2	1.54	1	1.54	7.68	0.0276[b]
X_3	2.31	1	2.31	11.53	0.0115[b]
$X_1 \times X_2$	0.67	1	0.67	3.36	0.1097
$X_1 \times X_3$	0.23	1	0.23	1.13	0.3239
$X_2 \times X_3$	0.12	1	0.12	0.59	0.4661
X_1^2	9.26	1	9.26	46.21	0.0003[a]
X_2^2	10.35	1	10.35	51.66	0.0002[a]
X_3^2	6.64	1	6.64	33.12	0.0007[a]
Residual	1.40	7	0.20		
Lack of fit	0.17	3	0.058	0.19	0.8997[c]
Pure error	1.23	4	0.31		
Cor total	35.91	16			
R^2	0.9609				
Adj R^2	0.9107				
Pred R^2	0.8694				
Adeq precision	11.570				
C.V. %	3.12				

[a] Significant at $p < 0.001$.
[b] Significant at $p < 0.05$.
[c] Not significant ($p > 0.05$).

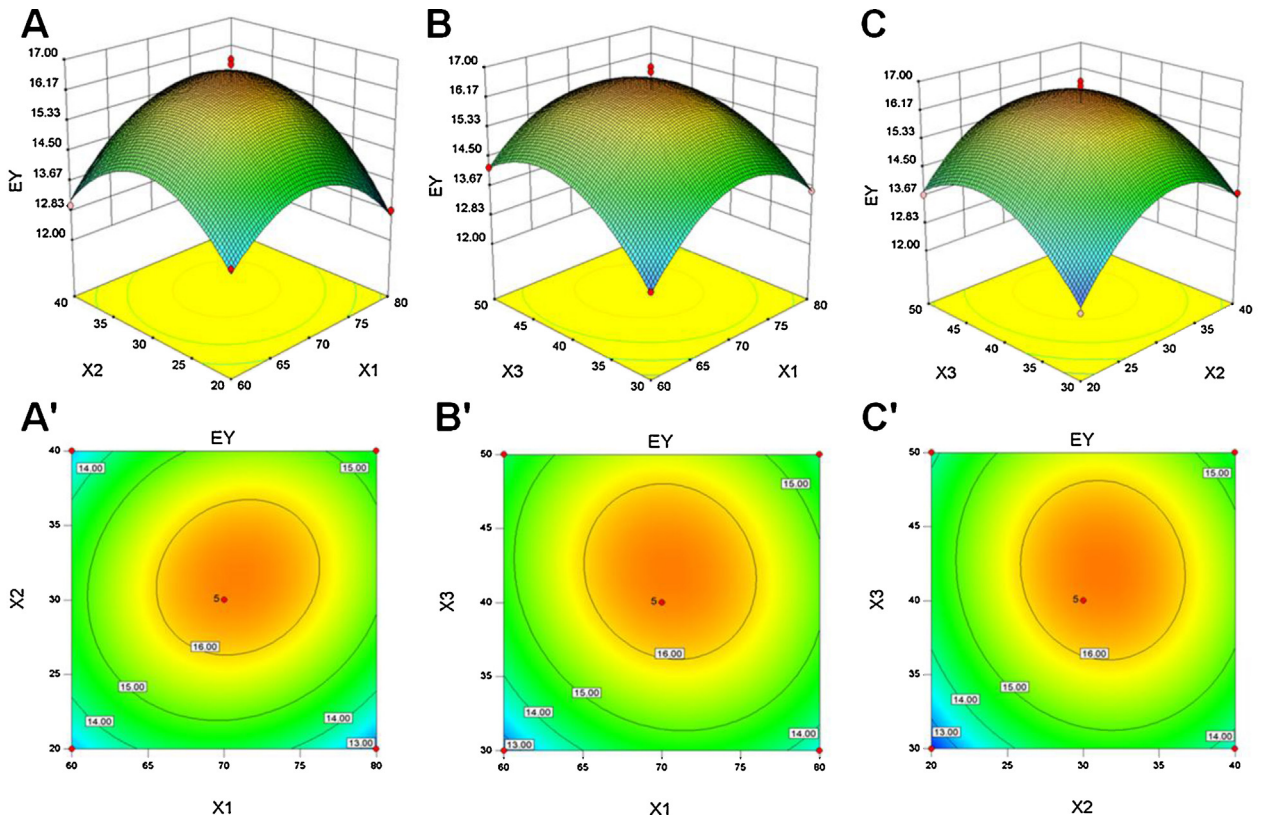

Fig. 3. Response surface plots (A, B, and C) and contour plots (A′, B′ and C′) showing the effect of variables (X_1: extraction temperature, °C; X_2: extraction time, min; and X_3: ratio of water to raw material, mL/g) on the extraction yield (EY) of BGPs.

stretching band (Gnanasambanda & Proctor, 2000), respectively, indicating that there were esterified and free carboxyl groups present in BGP. Besides, the peak absorption at 1417.58 cm^{-1} and 1070.42 cm^{-1} were due to carboxylate groups symmetric stretching and the pyranose ring, respectively.

3.3. Antioxidant activity assay of BGPs

3.3.1. Superoxide radical scavenging activity

The effects of BGPs were evaluated for its antioxidant activity on different *in vitro* models like scavenging of superoxide radical, ABTS

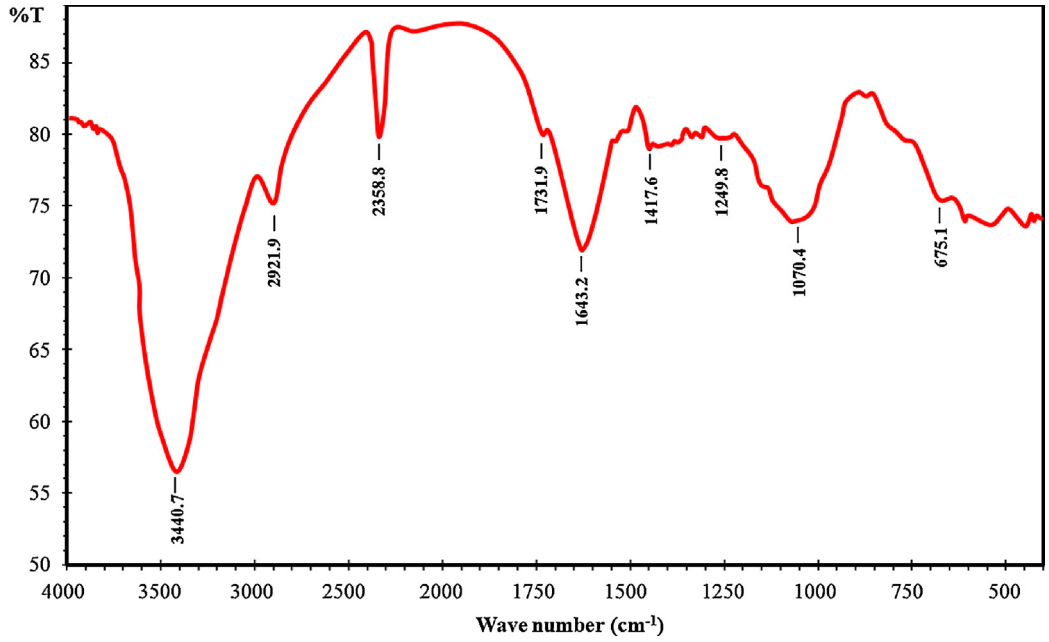

Fig. 4. FT-IR spectrum of BGPs.

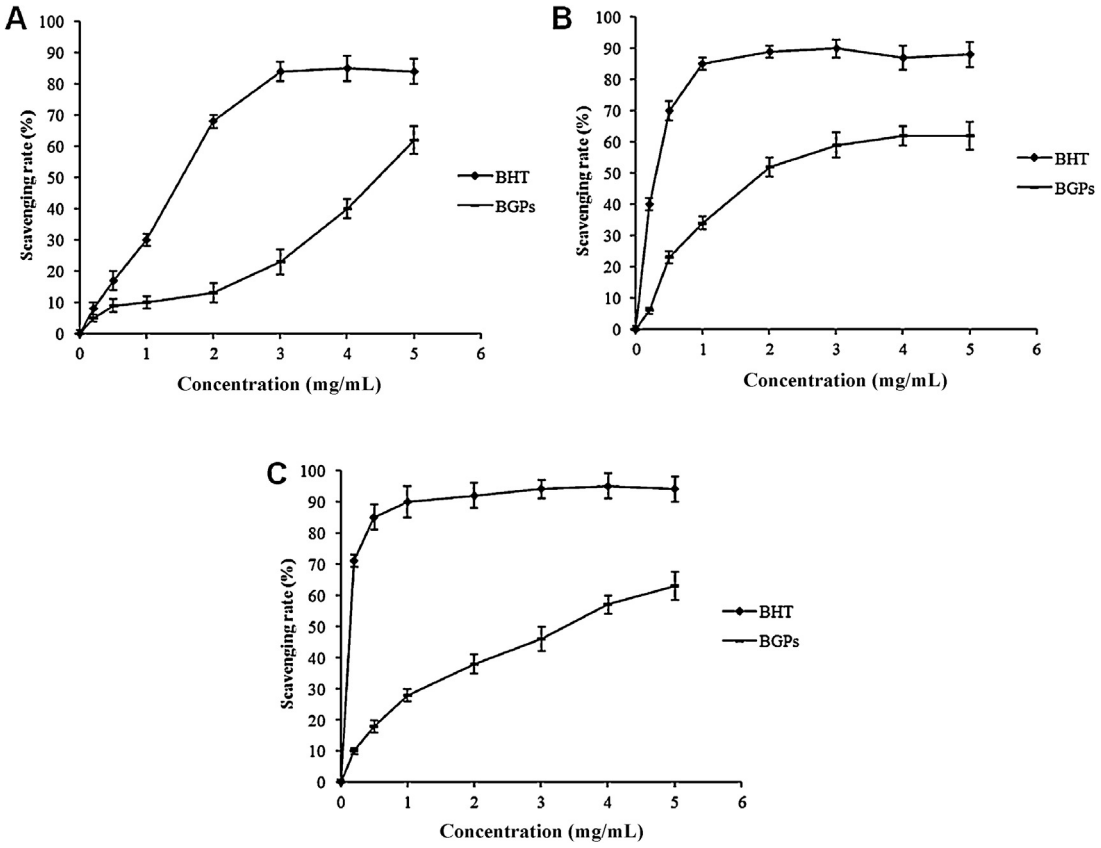

Fig. 5. Anti-oxidant effects of BGPs. Scavenging effects of BGPs on superoxide radical (A), ABTS radical (B) and hydroxyl radical (C). Values are means ± SD ($n = 3$).

radical scavenging activity, and hydroxyl radical-scavenging ability in a concentration dependent manner (Yuan, Zhang, Fan, & Yang, 2008; Chen et al., 2009; Zhang et al., 2009). Superoxide anion is one of the precursors of the singlet oxygen and hydroxyl radicals and the scavenging of superoxide radical is therefore important for the antioxidative defense (Marklund & Marklund, 1974). The results of superoxide radical scavenging assay are described in Fig. 5A. The scavenging activity increased with increase in the concentration of BGPs. Although the activity of BGPs was weaker than that of BHT, it can scavenge superoxide anion well in higher concentrations. At the concentration of 5 mg/mL, the scavenging capacity of BGPs was 62.4%, which was comparable to BHA (84.7%). Therefore, BGPs had an appreciable power on superoxide radicals.

3.3.2. *ABTS radical scavenging activity*

Within a short reaction time, the ABTS radical scavenging assay can well separated from the visible-light range with a maximum absorption at 734 nm and it has been widely applied to evaluating antioxidant activity (Wu et al., 2006). The scavenging effects of BGPs on the ABTS radical were measured and are shown in Fig. 5B. The scavenging ability of BGPs on ABTS radical was 34.6% at 1 mg/mL, and increased to 62.2% at 5 mg/mL in a concentration-dependent manner. This result indicated that the BGP was a good scavenger for ABTS radicals.

3.3.3. *Hydroxyl radical scavenging activity*

Hydroxyl radicals are one of representative reactive oxygen species generated in the body and are also thought to initiate cell damage *in vivo*. Thus, removing hydroxyl radicals is important for the protection of living systems (Yuan et al., 2008). As shown in Fig. 5C, the scavenging rate of hydroxyl radicals increased with increase in the concentration of BGPs, and was 63.3% at 5 mg/mL. But when the concentrations increased from 1.0 mg/mL to 5.0 mg/mL, BHT did not show any obvious increasing scavenging effects. The present result proved that BGP was a good scavenger for hydroxyl radical.

4. Conclusion

In the present study, the extraction process of BGPs was optimized by response surface methodology with a BBD design and investigated its activities. The optimal experimental extraction yield (16.43 ± 0.08)% was obtained when the extraction parameters as following conditions, extraction temperature at 71 °C, extracting time for 31.4 min, and ratio of water to raw material at 42. Under these optimum conditions, the experimental extraction yield of BGPs agreed closely with the predicted yield of 16.47%. Additionally, the results of antioxidant activity assay of BGPs showed that the polysaccharides had appreciable antioxidant activity and free radical-scavenging activity *in vitro*.

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