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Optimization of ultrasonic-assisted extraction and *in vitro* antioxidant activities of polysaccharides from *Trametes orientalis*

Yi Zheng[a,b,*], Yong Li[a], Wei-dong Wang[a]

[a] College of Food Engineering, Xuzhou Institute of Technology, Xuzhou 221000, China
[b] Jiangsu Key Construction Laboratory of Food Resource Development and Quality Safe, Xuzhou 221000, China

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ABSTRACT

A Box–Behnken design was employed to optimize ultrasonic-assisted extraction of *Trametes orientalis* polysaccharides (TOP). The crude polysaccharides were purified by DEAE cellulose-52 chromatography, giving a main fraction named as PTOP. The antioxidant properties of PTOP were evaluated by different *in vitro* antioxidant assays, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities, reducing power, superoxide radical scavenging activities, and chelating ability of ferrous ions. The results showed that optimal extraction parameters were as follows: ratio of water to raw material 30.6 mL/g, ultrasonic power 109.8 W, extraction temperature 40.2 °C, and extraction time 42.2 min. Under these conditions, the experimental yield of polysaccharides was 7.49 ± 0.14%, which agreed closely with the predicted value (7.47%). Furthermore, PTOP exhibited antioxidant capacity in a concentration-dependent manner in all assays.

1. Introduction

Mushroom polysaccharides have been reported to possess antitumor, immunomodulating, antioxidant, and antibacterial, and antibacterial activities (Lindequist, Niedermeyer, & Julich, 2005; Moradali, Mostafavi, Ghods, & Hedjaroude, 2007). These stunning properties, along with the absence of toxicity, render mushroom polysaccharides for the development of novel pharmaceutical products or functional foods (Giavasis, 2014; Wasser, 2002). Mushroom polysaccharides have shown widely inhibitory effects towards many kinds of tumors including Sarcoma 180 solid cancers, Ehrlich solid cancer, Sarcoma 37, Yoshida sarcoma and Lewis lung carcinoma (Zhang, Cui, Cheung, & Wang, 2007). The proposed mechanisms by which mushroom polysaccharides exert antitumor effect include: cancer-preventing activity; immuno-enhancing activity; and direct tumor inhibition activity (Leung, Liu, Koon, & Fung, 2006; Zong, Cao, & Wang, 2012).

It has been known that most bioactive mushroom polysaccharides belong to the family Polyporaceae. Some well-known polysaccharides from Polyporaceae, such as *Trametes* (*Coriolus*) *versicolor*, *Poria cocos*, *Polyporus umbellatus*, and *Ganoderma lucidum* have been in clinical use for many years in China. Nevertheless, only a small number of polysaccharides from the most common species of Polyporaceae have been studied (Zjawiony, 2004). Therefore, it might be more probable to screen successfully novel bioactive polysaccharides from Polyporaceae.

Trametes (*Polystictus*) *orientalis*, which belongs to *Trametes spp.* of Polyporaceae, is widespread in many districts of China, such as Jilin, Heilongjiang, Hubei, Jiangxi, Hunan, and Yunnan province. The modern pharmacology research indicated that it has the functions of antitumour (Ren, Liu, Zhu, Yang, & Fu, 2006) and antifungal activities (Tascioglu, Yalcin, Sen, & Akcay, 2013). *T. orientalis* is used in traditional Chinese medicine and believed to be beneficial to the lungs and other respiratory tissues (Chen & Chiang, 1995). However, the components responsible for such actions have not been clearly defined. Polysaccharides are the best known and most potent mushroom-derived substances with diverse biological properties. But there were few reports about polysaccharides from *T. orientalis* (TOP).

Extraction of polysaccharides is an essential process for further research and development. Recently, ultrasonic-assisted extraction (UAE), offering high yield at shorter times and lowered energy

Abbreviations: BBD, Box-Behnken design; DEAE, diethylaminoethanol; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EC_{50}, half maximal effective concentration; EDTA, ethylenediaminetetraacetic acid; NBT, nitroblue tetrazolium; PTOP, purified T. orientalis polysaccharides; ROS, reactive oxygen species; RSM, response surface methodology; SD, standard deviation; TOP, *Trametes orientalis* polysaccharides; UAE, ultrasonic-assisted extraction.

* Corresponding author at: College of Food Engineering, Xuzhou Institute of Technology, Xuzhou 221000, China. Tel.: +86 516 83105383; fax: +86 516 83105383.
E-mail addresses: biozheng@gmail.com (Y. Zheng), lishaoyong693@163.com (Y. Li), wwd.123@163.com (W.-d. Wang).

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input, has been widely employed to extract polysaccharides from different materials (Zou, Chen, Yang, & Liu, 2011; Pan et al., 2010; Xie et al., 2012; Chen, Wang, Zhang, & Huang, 2012). Box–Behnken design (BBD), one of response surface methodology (RSM), is more efficient and easier to arrange and interpret in experiments compared with other methods. It is widely used to optimize extraction process in various researches.

The objective of this study was to optimize the ultrasonic-assisted extraction conditions of polysaccharides from the fruiting bodies of *T. orientalis* using RSM. Effects of ratio of water to raw material, ultrasonic power, ultrasonic temperature, and ultrasonic time on the extraction yield of TOP were fully examined by BBD. In addition, we investigated the *in vitro* antioxidant of purified *T. orientalis* polysaccharides for seeking novel biological components used as pharmaceutical products and functional foods.

2. Materials and methods

2.1. Materials and chemicals

Fruiting bodies of *T. orientalis* were purchased from a wild products store (Huadian, China). *T. orientalis* originally grew in Changbai Mountain, Jilin province, China. 1,1-Diphenyl-2- picrylhydrazyl (DPPH), ascorbic acid, nitroblue tetrazolium (NBT), riboflavin, methionine, ferrozine, and DEAE-cellulose 52 were purchased from Sigma-Aldrich Co., LLC., USA. All other reagents were of analytical grade.

2.2. Ultrasonic-assisted extraction of polysaccharides

The dried fruiting bodies were ground to obtain a fine powder. The process of TOP extraction was conducted in an ultrasonic cell disintegrator (92-IID, Shanghai Bilon Instrument Co., Ltd., China). After the ultrasonic-assisted extraction, the extract solutions were centrifuged at 3000 r/min for 10 min and filtered to collect the supernatants. The insoluble residues were treated again for three times as described above. The supernatants were incorporated and concentrated using a rotary evaporator (R201L, Shanghai SENCO Technology Co., Ltd., China). The concentrates were precipitated by the addition of dehydrated ethanol to a final concentration of 80% (v/v) and incubated for 12 h at 4 °C. Then the precipitates were collected by centrifugation (3000 r/min for 10 min), washed with dehydrated ethanol, and lyophilized to obtain polysaccharides. The polysaccharides were determined using the phenol-sulfuric acid method with D-glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The TOP yield (%) was measured using the following equation:

$$\text{TOP yield}\,(\%) = \frac{\text{weight of dried crude TOP (g)}}{\text{weight of sample (g)}} \times 100 \qquad (1)$$

Hot water extraction of TOP without ultrasonic treatment was performed as a control experiment.

2.3. Experimental design

2.3.1. Single factor experimental design

The effects of ratio of water to raw material (15, 20, 25, 30 and 35 mL/g, respectively), ultrasonic power (60, 80, 100, 120 and 140 W, respectively), extraction temperature (20, 30, 40, 50, and 60 °C, respectively), and extraction time (10, 20, 30, 40 and 50 min, respectively) on TOP yield were studied by a single factor design as follows: one factor was changed while the other factors were kept constant in each experiment.

Table 1
Independent variables and their levels used for Box–Behnken design (BBD).

Independent variables	Symbol	Levels		
		−1	0	1
Ratio of water to raw material (mL/g)	X_1	25	30	35
Ultrasonic power (W)	X_2	80	100	120
Extraction temperature (°C)	X_3	30	40	50
Extraction time (min)	X_4	30	40	50

2.3.2. Box–Behnken design

A BBD with four independent variables (X_1, ratio of water to raw material; X_2, ultrasonic power; X_3, extraction temperature; and X_4, extraction time) at three levels was performed, as shown in Table 1. The ranges of independent variables were chosen based on the single factor experiment results.

Extraction yield (Y) was taken as the response of the design experiments. Experimental data were analyzed by multiple regressions to fit the following quadratic polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{4}\beta_i X_i + \sum_{i<j}^{3}\sum_{j}^{4}\beta_{ij} X_i X_j + \sum_{i=1}^{4}\beta_{ii} X_i^2 \qquad (2)$$

where Y is the predicted response and β_0 is an intercept. β_i, β_{ij}, and β_{ii} are regression coefficients for linear, quadratic, and interactive terms, respectively. X_i and X_j are the coded independent variables, respectively.

2.4. Preparation and purification of polysaccharides

The crude TOP were resolved for the removal of proteins utilizing the Sevag method (Sevag, Lackman, & Smolens, 1938) and purified by DEAE-52 anion-exchange chromatography according to the reported method (Ye, Wang, Zhou, Liu, & Zeng, 2008) with some modifications. Briefly, 2 mL of polysaccharides solution (10 g/L) were applied to a column of DEAE cellulose-52 (2.6 × 30 cm), followed by stepwise elution with 0, 0.1, 0.3, and 0.5 mol/L sodium chloride solutions at a flow rate of 60 mL/h. The obtained eluates (10 mL/tube) were collected and the carbohydrates were determined by the phenol-sulfuric acid method, using glucose as standard. Finally, the main fraction (PTOP) was obtained, dialyzed, and lyophilized for investigation of antioxidant activities.

2.5. Carbohydrate, protein, and uronic acid analysis

Carbohydrate content of PTOP was measured by phenol-sulfuric acid, using D-glucose as a standard. Protein content was determined according to the method of Bradford (1976) with bovine serum albumin as a standard. Uronic acid content of purified fractions was determined by m-hydroxydiphenyl with galacturonic acid as a standard (Blunenkrantz & Asboe-Hansen, 1973).

2.6. Antioxidant activity analysis in vitro

2.6.1. DPPH radical scavenging assay

The scavenging effect of the PTOP on DPPH radical was measured by the method of Chen, Xie, Nie, Li, and Wang (2008) with some modifications. 1.5 mL of sample was in addition to 1.5 mL of 0.1 mmol/L DPPH in 95% (v/v) ethanol. The mixture was shaken and kept in dark place for 30 min at room temperature; afterwards the absorbance of the resulting solution was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Ascorbic acid was used as a positive

control. The scavenging rate was calculated according to the following equation:

$$\text{scavenging rate}\,(\%) = \frac{A_c - (A_i - A_j)}{A_c} \times 100$$

where A_c is the absorbance of DPPH solution without sample, A_i is the absorbance of the test sample mixed with DPPH solution, and A_j is the absorbance of the sample without DPPH solution.

2.6.2. Measurement of reducing power

The reducing power of PTOP was determined according to the method of Oyaizu (1986). Various concentrations of samples (2 mL) were mixed with 2 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2 mL of 10 g/L potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then 2 mL of 100 g/L trichloroacetic acid was added to the reaction mixture. Afterwards, the mixture was centrifuged at 3000 r/min for 10 min. Subsequently, 2 mL of supernatant from each incubated mixture was mixed with 2 mL of deionized water and 0.4 ml of 1 g/L ferric chloride in a test tube. After a 10 min reaction at 50 °C, the absorbance of the resulting solution was measured at 700 nm. Higher absorbance suggests higher reducing power. Ascorbic acid was used as a positive control.

2.6.3. Superoxide radical scavenging assay

The superoxide radical scavenging activity of PTOP was determined by the method of Wang et al. (2011) with some modifications. Reaction mixture contained 130 mmol/L methionine, 20 μmol/L riboflavin, 750 μmol/L NBT, 20 μmol/L ethylenediaminetetraacetic acid (EDTA), 0.05 mol/L phosphate buffer (pH 7.8), and the PTOP. After illuminating the reaction mixture with a fluorescent lamp at 25 °C for 30 min, the absorbance of the PTOP was measured at 560 nm, using ascorbic acid for a positive control. The scavenging rate of PTOP on superoxide radical was calculated using the following equation:

$$\text{scavenging rate}\,(\%) = \frac{A_b - A_s}{A_b} \times 100$$

where A_b is the absorbance of the control (phosphate buffer, instead of sample) and A_s is the absorbance of the test sample mixed with the reaction solution.

2.6.4. Chelating ability of ferrous ions

Chelating activity of PTOP on Fe^{2+} was measured as reported previously (Sun, Liu, & Kennedy, 2010) with some modifications. 2 mL of sample was mixed with 3.7 mL of deionized water, and then reacted with ferrous chloride (5 mmol/L, 0.1 mL). After 0.2 mL of 5 mmol/L ferrozine was added, the solution was mixed, left for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm. EDTA was employed as a positive control. A lower level of absorbance indicated stronger chelating activity. The chelating rate of PTOP on Fe^{2+} (%) was calculated according to the following equation:

$$\text{chelating rate}\,(\%) = \frac{A_b - A_s}{A_b} \times 100$$

where A_b is the absorbance of the control (deionized water, instead of sample), and A_s is the absorbance of the test sample mixed with the reaction solution.

3. Results and discussion

3.1. Single factor experiment

3.1.1. Effect of different ratio of water to raw material on the yield of polysaccharides

The effect of ratio of water to raw material on extraction yield of polysaccharides is shown in Fig. 1a. Extraction was carried out at different ratio of water to raw material (15, 20, 25, 30 and 35 mL/g), when other extraction parameters were as follows: ultrasonic power of 100 W, extraction temperature of 40 °C, and extraction time of 20 min. The yield of polysaccharides increased rapidly when the ratio of water to raw material ranged from 15 to 30 mL/g, and thereafter there was a little rise in the yield of polysaccharides as the ratio continued to increase. To avoid wasting consumption of solvents, 30 mL/g was chosen as the optimum ratio of water to raw material for polysaccharides production.

3.1.2. Effect of different ultrasonic power on the yield of polysaccharides

Fig. 1b lists the effect of ultrasonic power on the yield of polysaccharides. The other parameters were fixed as follows: ratio of water to raw material of 20 mL/g, extraction temperature of 40 °C, and extraction time of 20 min. It was found that the extraction yield of polysaccharides was significantly increased with increasing ultrasonic power, reaching the peak value at 120 W. The yield of polysaccharides decreased after 120 W, which might be because excessive ultrasonic power could result in the degradation of polysaccharides (Li, Wang, Wang, Walid, & Zhang, 2012). Therefore, 120 W was chosen as the optimum ultrasonic power.

3.1.3. Effect of different extraction temperature on the yield of polysaccharides

The effect of extraction temperature on the yield of polysaccharides was investigated with ratio of water to raw material of 20 mL/g, ultrasonic power of 100 W, and extraction time of 20 min. As shown in Fig. 1c, the yield of polysaccharides increased sharply when the extraction temperature increased from 20 to 40 °C, and declined gradually with further increasing extraction temperature. High temperature decreased the number of cavitation bubbles and weakened the impact of cavity collapse on ground samples (Zhao, Kwok, & Liang, 2007). Thus, the yield of polysaccharides decreased when the extraction temperature was over 40 °C. Accordingly, the optimum extraction temperature was 40 °C.

3.1.4. Effect of different extraction time on the yield of polysaccharides

The effect of extraction time on the yield of polysaccharides (Fig. 1d) was examined with ratio of water to raw material of 20 mL/g, ultrasonic power of 100 W, and extraction temperature of 40 °C. The results showed that the yield of polysaccharides considerably increased during the initial 40 min. Further increase in extraction time induced the degradation of polysaccharides, and then the yield of polysaccharides decreased (Ying, Han, & Li, 2011). Consequently, the optimum extraction time was 40 min.

3.2. Optimization of extraction of TOP by RSM

3.2.1. Statistical analysis and the model fitting

Based on the results of single factor experiments, a 29-run BBD was utilized to optimize the four independent variables, including X_1 (ratio of water to raw material), X_2 (ultrasonic power), X_3 (extraction temperature), and X_4 (extraction time). Table 2 shows the experimental conditions and the extraction yields of TOP.

Design-Expert (Version 8.06) software was adopted to analyze the experimental data. The results revealed that the response

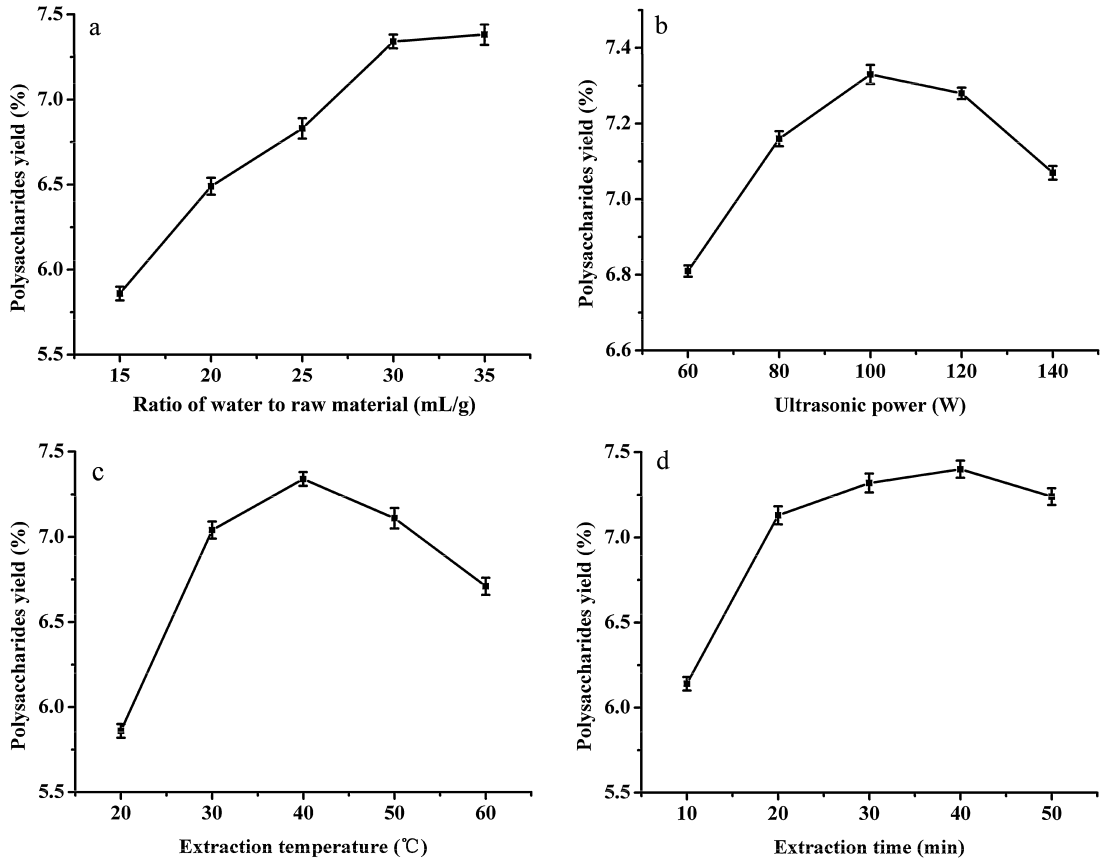

Fig. 1. Effect of different extraction parameters ((a) ratio of water to raw material, mL/g; (b) ultrasonic power, W; (c) extraction temperature, °C, and (d) extraction time, min) on yield of polysaccharides.

Table 2
The Box–Behnken design matrix and the results for extraction yield of TOP.

Run	X_1 (ratio of water to raw material, mL/g)	X_2 (ultrasonic power, W)	X_3 (extraction temperature, °C)	X_4 (extraction time, min)	TOP yield (%)
1	1(35)	0(100)	1(50)	0(40)	7.20
2	0(30)	−1(80)	0(40)	1(50)	6.93
3	0(30)	0(100)	−1(30)	−1(30)	6.94
4	1(35)	−1(80)	0(40)	0(40)	7.03
5	−1(25)	−1(80)	0(40)	0(40)	6.95
6	−1(25)	0(100)	−1(30)	0(40)	6.91
7	1(35)	0(100)	−1(30)	0(40)	7.06
8	0(30)	0(100)	0(40)	0(40)	7.42
9	0(30)	−1(80)	−1(30)	0(40)	6.83
10	0(30)	−1(80)	0(40)	−1(30)	6.97
11	0(30)	1(120)	0(40)	−1(30)	7.16
12	0(30)	1(120)	−1(30)	0(40)	7.37
13	0(30)	0(100)	0(40)	0(40)	7.50
14	−1(25)	0(100)	1(50)	0(40)	7.11
15	0(30)	0(100)	0(40)	0(40)	7.43
16	1(35)	0(100)	0(40)	1(50)	7.28
17	0(30)	0(100)	0(40)	0(40)	7.39
18	0(30)	0(100)	0(40)	0(40)	7.42
19	−1(25)	1(120)	0(40)	0(40)	7.24
20	0(30)	1(120)	1(50)	0(40)	7.23
21	0(30)	0(100)	1(50)	1(50)	7.21
22	1(35)	1(120)	0(40)	0(40)	7.21
23	0(30)	0(100)	−1(30)	1(50)	7.11
24	0(30)	−1(80)	1(50)	0(40)	7.25
25	1(35)	0(100)	0(40)	−1(30)	6.90
26	−1(25)	0(100)	0(40)	1(50)	6.98
27	0(30)	1(120)	0(40)	1(50)	7.31
28	−1(25)	0(100)	0(40)	−1(30)	7.12
29	0(30)	0(100)	1(50)	−1(30)	7.26

Table 3
Analysis of variance (ANOVA) for response surface quadratic model.

Source	Sum of squares	*df*	Mean square	*F*-value	Prob> *F*	Significance
Model	0.9538	14	0.0681	36.7007	<0.0001	***
X_1	0.0115	1	0.0114	6.1457	0.0265	*
X_2	0.2028	1	0.2028	109.2490	< 0.0001	***
X_3	0.0902	1	0.0901	48.5551	< 0.0001	***
X_4	0.01848	1	0.0184	9.9166	0.0071	**
X_1X_2	0.0030	1	0.0030	1.6296	0.2225	****
X_1X_3	0.0009	1	0.0009	0.4848	0.4976	****
X_1X_4	0.0676	1	0.0676	36.4163	< 0.0001	***
X_2X_3	0.0784	1	0.0784	42.2343	< 0.0001	***
X_2X_4	0.0090	1	0.0090	4.8618	0.0447	*
X_3X_4	0.0121	1	0.0121	6.5183	0.0230	*
X_1^2	0.2567	1	0.2567	138.2616	< 0.0001	***
X_2^2	0.1229	1	0.1229	66.2242	< 0.0001	***
X_3^2	0.1229	1	0.1229	66.2242	< 0.0001	***
X_4^2	0.2019	1	0.2019	108.7523	< 0.0001	***
Residual	0.0260	14	0.0019			
Lack of fit	0.0193	10	0.0019	1.1562	0.4827	****
Pure error	0.0067	4	0.0017			
Cor total	0.9798	28				
R^2	0.9735					
R^2_{adj}	0.9470					
Adeq precision	20.40					

**** Non-significant, $p > 0.05$.
* Significant, $p < 0.05$.
** Very significant, $p < 0.01$.
*** Highly significant, $p < 0.001$.

variable and the independent variables were related by the following second-order polynomial equation:

$$Y = 7.43 + 0.031X_1 + 0.13X_2 + 0.087X_3 + 0.039X_4 - 0.027X_1X_2 - 0.015X_1X_3 + 0.13X_1X_4 - 0.14X_2X_3 + 0.047X_2X_4 - 0.055X_3X_4 - 0.20X_1^2 - 0.14X_2^2 - 0.14X_3^2 - 0.18X_4^2 \quad (3)$$

where Y represents the yield of TOP (%); X_1, X_2, X_3 and X_4 represent ratio of water to raw material, ultrasonic power, extraction temperature, and extraction time, respectively. The analysis of variance is shown in Table 3.

As shown in Table 3, the model *F*-value of 36.7007 implied the model was significant. Meanwhile, the value of the determination coefficient ($R^2 = 0.9735$) and the adjusted determination coefficient ($R^2_{Adj} = 0.9470$) also confirmed that the model was statistically significant. Additionally, the lack of fit was not significant relative to the pure error. Adeq precision measured the signal to noise ratio and a ratio greater than 4 is desirable. Thus, ratio of 20.396 indicated an adequate signal. In conclusion, the model was found to be adequate for navigating the design space.

The *p*-value is used as a tool to check the significance of each coefficient, which in turn indicates the interaction strength between each independent variable. It could be observed that the coefficients of X_2, X_3, X_1X_4, X_2X_3, X_1^2, X_2^2, X_3^2, and X_4^2 were highly significant ($p < 0.001$). Meanwhile, the coefficients of X_1, X_2X_4, and X_3X_4 were found to be significant ($p < 0.05$). Moreover, the coefficients of X_1X_2, X_1X_3 were found non-significant ($p > 0.05$).

3.2.2. Optimization of TOP extraction conditions

The contour plots and 3D response surface plots, obtained by the Design Expert software, shown in Figs. 2 and 3, were the graphical representations of the regression Eq. (3). In the contour plots and response surface plots, the extraction yield of TOP was obtained along with two continuous variables, while the other two variables were fixed constant at their respective 0 level. In the two figures, the maximum predicted value indicated by the surface was confined in the smallest ellipse in the contour diagram. Elliptical contours are obtained when there is a perfect interaction between the independent variables (Zhong & Wang, 2010).

The TOP yield affected by the ratio of water to raw material and ultrasonic power is seen in Figs. 2a and 3a, when extraction temperature and extraction time were maintained at 0 level. It was obvious that the TOP yield increased as the ratio of water to raw material was extended from 25 to 31 mL/g. Subsequently, the TOP yield gradually decreased. The yield also increased as ultrasonic power increased from 80 to 109 W; the TOP yield slightly reduced after ultrasonic power of 109 W. Figs. 2b and 3b show the TOP yield at varying ratio of water to raw material and extraction temperature, which indicated that the interaction effect between these two factors was not significant. The maximum TOP yield was obtained when the ratio of water to raw material and extraction temperature were 31 mL/g and 109 W, respectively. The TOP yield affected by the ratio of water to raw material and extraction time is shown in Figs. 2c and 3c, whereas the two variables (ultrasonic power and extraction temperature) were fixed at 0 level. The maximum TOP yield was obtained when the ratio of water to raw material and extraction time were 30 mL/g and 40 min, respectively. The TOP yield affected by the ultrasonic power and extraction temperature is seen in Figs. 2d and 3d. The maximum TOP yield was obtained when the ultrasonic power and extraction temperature were 109 W and 40 °C, respectively. Figs. 2e and 3e indicated that the maximum TOP yield could be achieved when ultrasonic power and extraction time at the threshold level of 109 W and 40 min, respectively. The TOP yield affected by extraction temperature and extraction time is seen in Figs. 2f and 3f, when other two variables (ratio of water to raw material and ultrasonic power) were fixed at 0 level. It could be seen that the TOP yield increased as the extraction temperature was increased from 20 to 40 °C, then declined gradually with further increasing extraction temperature. It was also found that the TOP yield was significantly increased with increasing extraction time, reaching the peak value at 40 min.

3.2.3. Verification of predictive model

The optimal extraction conditions for achieving maximal yield of TOP obtained by the regression Eq. (3) were as follows: ratio of water to raw material 30.6 mL/g, ultrasonic power 109.8 W, extraction temperature 40.2 °C, and extraction time 42.2 min. The predicted yield value was 7.47%. Under optimal extraction

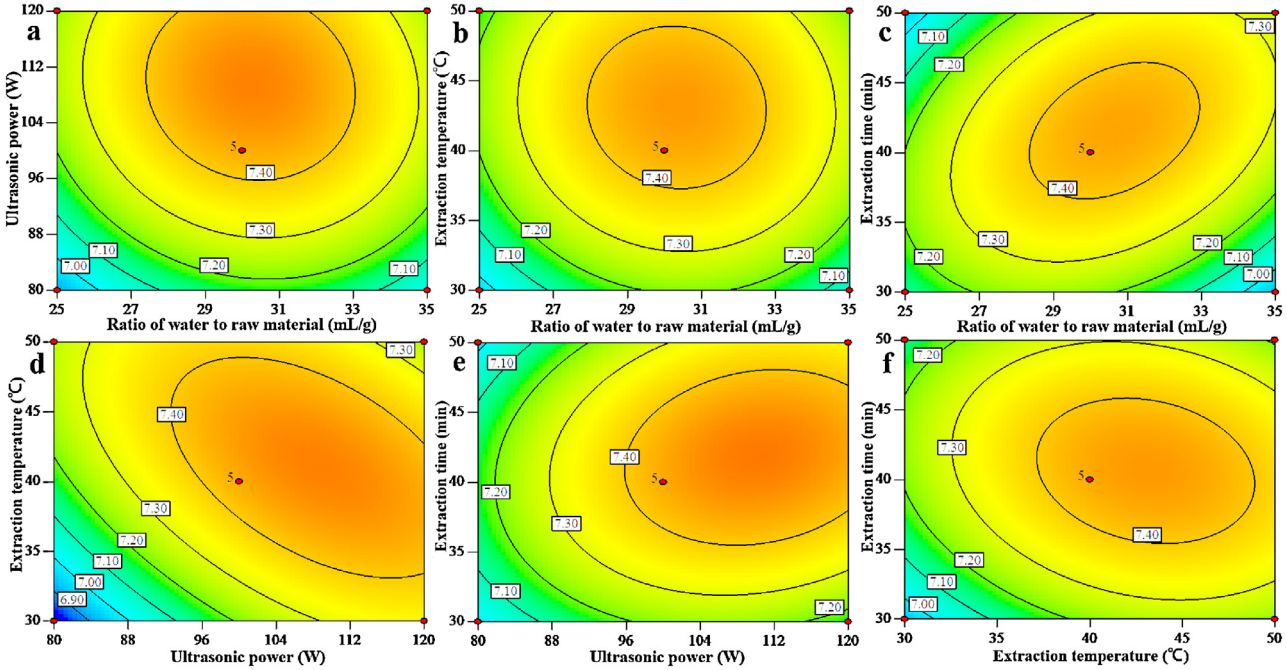

Fig. 2. Contour plots showing the interactive effects of ratio of water to raw material and ultrasonic power (a), ratio of water to raw material and extraction temperature (b), ratio of water to raw material and extraction time (c), ultrasonic power and extraction temperature (d), ultrasonic power and extraction time (e), extraction temperature and extraction time (f) on yield of polysaccharides.

conditions, the experimental yield of TOP was 7.49 ± 0.14% (n = 3), which was well-matched with the predicted value, indicating that the model was adequate for the extraction process.

The TOP yield was also examined by hot water extraction with ratio of water to raw material of 30.6 mL/g and extraction temperature of 80 °C. The TOP yield decreased from 6.32% to 6.03% when extraction time changed from 120 to 150 min. The results showed that lengthening extraction time excessively at high temperature might induce the degradation of polysaccharides and therefore lead to the decline of the TOP yield. Compared with hot water extraction of TOP, UAE technique employed for the extraction of TOP in this study was time-saving, energy-saving and gave a higher yield.

3.3. Purification of *T. orientalis* polysaccharides

T. orientalis polysaccharides were purified by DEAE cellulose-52 chromatography resulting in a main elution peak (Fig. 4). The main fraction named as PTOP, was eluted with 0.1 mol/L sodium chloride solution. Then PTOP was collected, dialyzed with water and

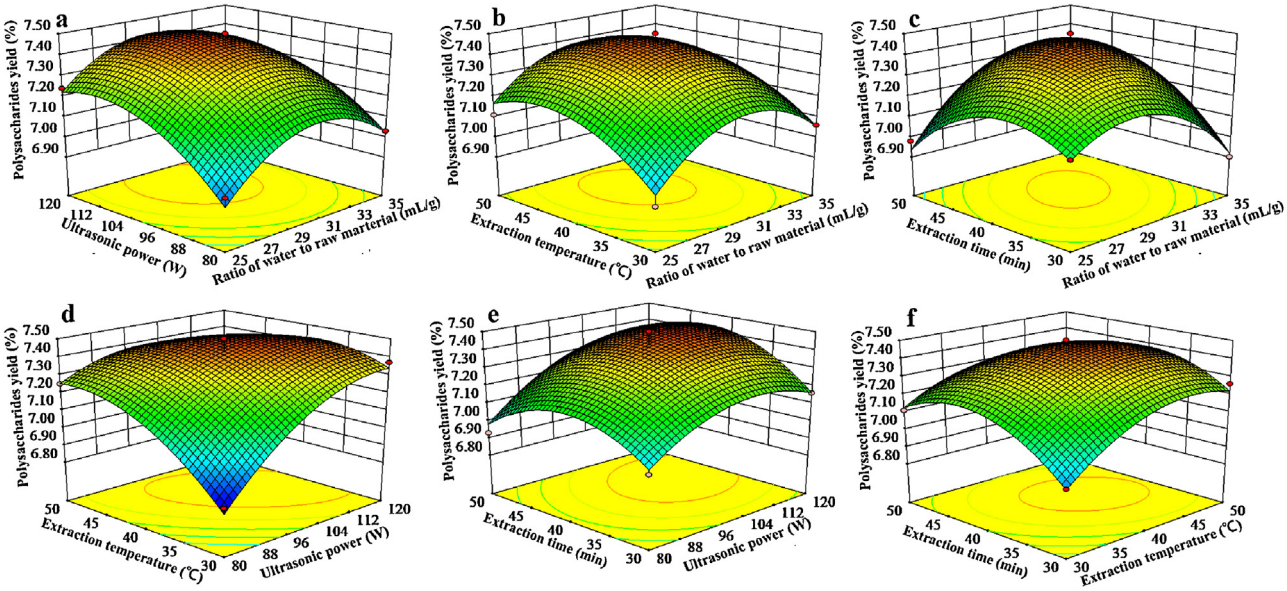

Fig. 3. 3D-response surface plots showing the interactive effects of ratio of water to raw material and ultrasonic power (a), ratio of water to raw material and extraction temperature (b), ratio of water to raw material and extraction time (c), ultrasonic power and extraction temperature (d), ultrasonic power and extraction time (e), extraction temperature and extraction time (f) on yield of polysaccharides.

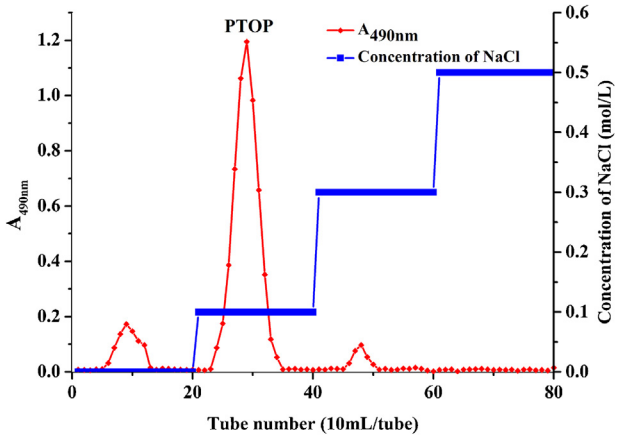

Fig. 4. Elution curve of *T. orientalis* polysaccharides by DEAE Cellulose-52 anion-exchange chromatography.

lyophilized for further study of antioxidant activities. Moreover, the yield of PTOP was 62.74% based on crude polysaccharides.

Carbohydrate contents in TOP and PTOP were 70.63 ± 3.8% and 91.57 ± 5.1%, respectively. The protein content of TOP was 2.37 ± 0.11%, and no protein was detected in PTOP, indicating that the purification of crude TOP may be useful and efficient. Furthermore, uronic acid content of PTOP was 1.87 ± 0.07%, suggesting that PTOP was acid polysaccharide.

3.4. Antioxidant activity assay

3.4.1. DPPH radical scavenging assay

DPPH radical scavenging assay, based on the reduction of DPPH solution in the presence of a proton-donating substance, has been extensively employed to evaluate the free radical scavenging ability of varied samples (Chen, Ma, Liu, Liao, & Zhao, 2012). The scavenging ability of PTOP on DPPH radical is shown in Fig. 5a and compared with ascorbic acid as a control standard. Fig. 5a illustrates that the scavenging abilities of PTOP and ascorbic acid are in a concentration-dependent manner. At the concentration of 0.5 g/L, PTOP showed scavenging rate of 36.34 ± 0.9% on DPPH radicals, and at 3 g/L, scavenging rate increased to 82.97 ± 2%.

The half maximal effective concentration (EC_{50}) is defined as the concentration of the sample at which the scavenging rate reaches 50%. Practically, a lower EC_{50} value corresponds to stronger antioxidant activity of tested sample (Kozarski, Klaus, Niksic, Jakovljevic, Helsper, & Van Griensven, 2011). The EC_{50} value of DPPH radical scavenging activity for PTOP, calculated by Origin Pro (Version 8.6), was 0.87 g/L, which indicated that PTOP had a noticeable effect on scavenging DPPH radical. However, scavenging ability of PTOP was lower than that of ascorbic acid at all investigated concentrations.

3.4.2. Reducing power

In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. It has been reported that the reducing power is positively related to the antioxidant activities (Fan, Li, Deng, & Ai, 2012). The reducing power of

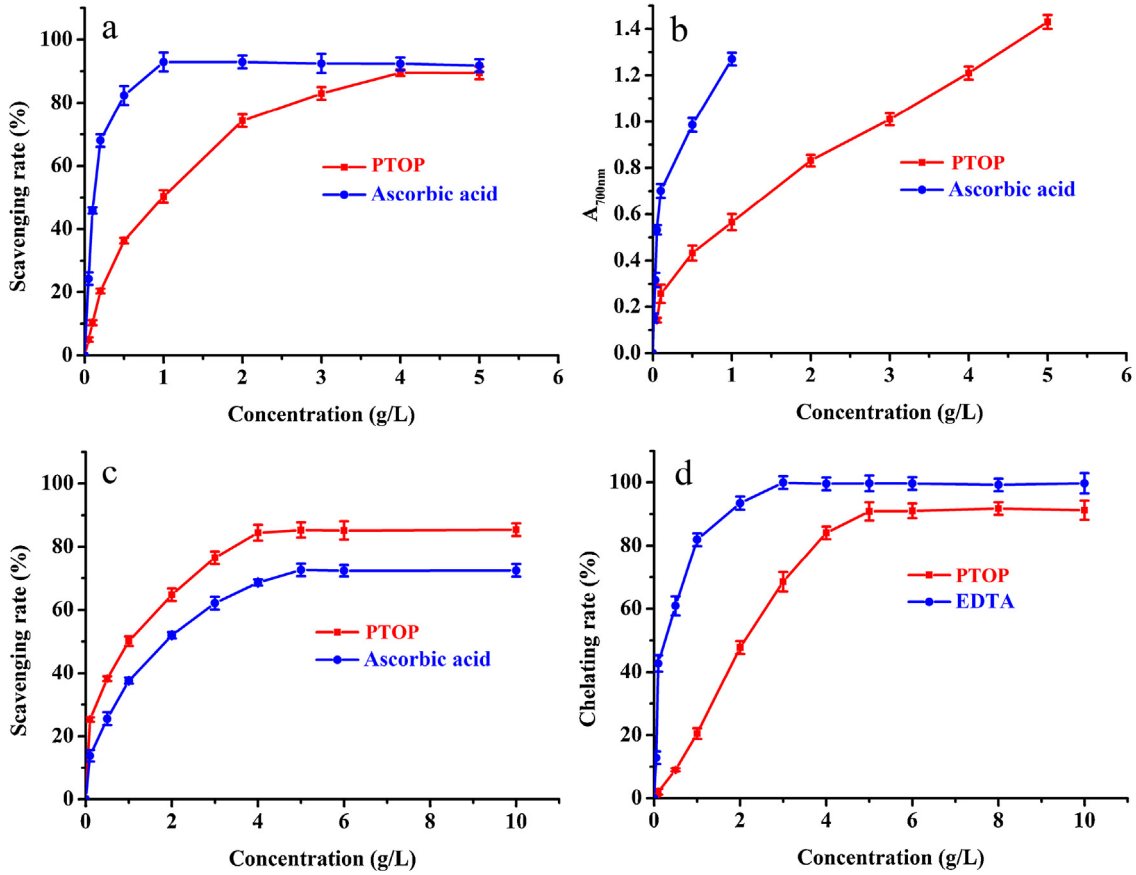

Fig. 5. Antioxidant activities of PTOP and ascorbic acid: (a) DPPH radical scavenging activities, (b) reducing power, (c) superoxide radical scavenging activities and (d) chelating abilities of PTOP and ascorbic acid.

PTOP and ascorbic acid determined at 700 nm is depicted in Fig. 5b. In the range of 0.05 to 3.0 g/L, the reducing power of PTOP increased from 0.142 to 1.012. Ascorbic acid, used as a positive control, had a reducing power of 1.271 at 1.0 g/L. It showed that the reducing power of PTOP was significantly lower than that of ascorbic acid. Owing to the fact that the absorbance should be lower than 1 for preventing unacceptable relative errors, we did not further augment the tested concentration. The data of reducing power suggested that PTOP could act as electron donors and can react with free radicals to convert them to more stable products.

3.4.3. Superoxide radical scavenging assay

Superoxide radical, a relatively weak oxidant, considered as the primary ROS, can further interact with other molecules to generate secondary ROS such as hydrogen peroxide, hydroxyl radical that result in oxidative damage in lipids, proteins and DNA (Wu, Hu, Li, Huang, & Jiang, 2014). As shown in Fig. 5c, it was obvious that the scavenging ability of PTOP on superoxide radical positively correlated with increasing concentrations. Scavenging rates of PTOP on superoxide radical were $38.22 \pm 0.8\%$, $50.14 \pm 2\%$, and $84.46 \pm 1\%$ at the concentration of 0.5, 1.0, and 5.0 g/L, respectively. Additionally, PTOP demonstrated more powerful scavenging activities on superoxide radical than ascorbic acid. The EC_{50} values of superoxide radical scavenging activity for PTOP and ascorbic acid were 0.79 and 1.65 g/L, respectively. Depending on the EC_{50} values, PTOP also had better scavenging effects on superoxide radical than ascorbic acid.

3.4.4. Chelating ability on ferrous ions

Chelating of transition metal ions, such as $Fe^{2+}$and Cu^{2+}, which could trigger a process of free radical reaction and result in lipid per-oxidation and DNA damage, would retard the oxidation reaction. Fe^{2+}, one of the most powerful pro-oxidant, can quantitatively form red complexes with ferrozine (Chen, Ju, Li, & Yu, 2012). In the presence of chelating agent, such as EDTA and antioxidant, the red color of the complexes becomes lighter due to disrupted complex formation. Chelating abilities of PTOP and EDTA on ferrous ions are shown in Fig. 5d. At the concentration of 1.0 g/L, PTOP showed chelating ability of $20.50 \pm 0.5\%$ on ferrous ions, whereas at 2.0 g/L and 5.0 g/L, the chelating rates were $47.49 \pm 2\%$ and $90.84 \pm 3\%$, respectively. Nevertheless, EDTA showed superior chelating ability of $93.46 \pm 2\%$ at 2 g/L. EC_{50} value of chelating activity on ferrous ions of PTOP was 1.98 g/L, while that of EDTA was 0.22 g/L.

4. Conclusion

An efficient ultrasonic-assisted extraction technique was employed to extract polysaccharides from the fruit bodies of *T. orientalis*. A Box–Behnken design was employed to optimize extraction parameters. The optimal conditions determined were as follows: ratio of water to raw material 30.6 mL/g, ultrasonic power 109.8 W, extraction temperature 40.2 °C, and extraction time 42.2 min. Under these conditions, the experimental yield of TOP was $7.49 \pm 0.14\%$, which agreed closely with the value (7.47%) predicted by RSM model. Polysaccharides from *T. orientalis* exhibited potent antioxidant activities in a concentration-dependent manner. It suggests that PTOP may be developed as a naturally potential antioxidant for nutraceutical and pharmaceutical, while characterization, *in vivo* antioxidant activities and the antioxidant mechanism of PTOP need to be elucidated.

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