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Optimum extraction of polysaccharides from *Opuntia dillenii* and evaluation of its antioxidant activities

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ABSTRACT

Response surface methodology (RSM) was applied to optimize the extraction of crude polysaccharides from *Opuntia dillenii* (Ker–Gaw) Haw. A three-level, four-variable Box–Behnken design was employed to obtain the best possible combination of extraction temperature (80–90 °C), extraction time (50–70 min), number of extraction cycle (1–3 times), and ratio of water to raw material (8:1–12:1, v/w) for maximum yield of crude polysaccharide. Besides, the antioxidant capacity of crude polysaccharide was evaluated by DPPH assay. The results showed that optimized extraction conditions were extraction temperature 85 °C, extraction time 63.7 min, extraction 2 times and ratio of water to raw material 11.14:1. Under these conditions, the experimental yield was 27.36 ± 0.21%, which is well in close agreement with the value (27.44%) predicted by RSM model. Pharmacological test showed that *O. dillenii* crude polysaccharides had a good antioxidant activity.

1. Introduction

Opuntia dillenii (Ker–Gaw) Haw, called Xian Ren Zhang in China, belongs to family *Opuntia* and usually grows in semi-desert regions in tropics and subtropics (Labra et al., 2003; López, de Ita, & Vaca, 2009). Since the early 20th century, *O. dillenii* has been used for livestock feed and alcohol production (Gimeno & Vilà, 2002; Obón, Castellar, Alacid, & Fernández-López, 2009). Thereafter, its medicinal value is heeded and investigated extensively. In recent years, the roots and stems of *O. dillenii* have been used in Chinese folk medicine to treat acute mastitis, mumps, boils and carbuncles (Chang, Hsieh, & Yen, 2008; Liu et al., 2009; Loro, del Rio, & Pérez-Santana, 1999; Morales, Ramírez-Moreno, Sanchez-Mata, Carvalho, & Ferreira, 2012). Furthermore, its effectiveness to dysentery, stomach and duodenal ulcers, bronchial asthma, diabetes was reported (Paulsen & Lund, 1979; Sáenz, Sepúlveda, & Matsuhiro, 2004; Schepetkin et al., 2008; Valente et al., 2010).

Cactus polysaccharide, extracted from *O. dillenii*, has been proved to be a biologically active ingredient. It is reported that cactus polysaccharide can enhance immunity by improving thymus exponent, spleen exponent and the rate of lymphocyte transformation (Alimi, Hfaiedh, Bouoni, Sakly, & Ben Rhouma, 2011; Huang, Li, Li, & Guo, 2009; Liang, Liu, & Cao, 2008). Many studies have proved that cactus polysaccharide has an anti-fatigue and anti-inflammatory effect (Liu et al., 2009; Schepetkin et al., 2008; Valente et al., 2010), and cactus polysaccharide is believed to have the function of enhancing the body's resistance to the non-specific stimuli and the effect of reducing the damage caused by inflammatory cytokines (Zhao, Lan, Huang, Ouyang, & Zeng, 2011; Zhao, Zhang, Yuan, Cheng, & Zeng, 2012). Besides, it is found that cactus polysaccharide can reduce the content of serum malondialdehyde (MDA) and lipofuscin (Lf) in brain and liver tissue of old rat (Loro et al., 1999; Valente et al., 2010; Zhao et al., 2012).

Cactus polysaccharide has attracted much interest because of its multiple important pharmacological actions. So optimizing extraction process to get maximum polysaccharides content is becoming a research focus in recent years (Chang et al., 2008; Gan & Latiff, 2011; Gan, Abdul Manaf, & Latiff, 2010; Göksungur, Uzunoğulları, & Dağbağlı, 2011; Qiu, Chen, Pei, Matsuda, & Yoshikawa, 2002; Zhao et al., 2011). Response surface methodology (RSM), as an effective statistical method, is widely used for optimizing complex processes since it can depict the complete effects of variables, and it is less laborious and time-consuming (Chen et al., 2013; Hong, Liu, Li, & She, 2013; Prakash Maran, Manikandan, Thirugnanasambandham, Vigna Nivetha, & Dinesh, 2013). In this paper, in order to obtain higher extraction yield and basic technological information for the OCP, the approach of response surface was used. The extraction parameters studied were extraction temperature, extraction time, number of extraction cycle and ratio of water to raw material. Furthermore, DPPH assay was used to

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Table 1
The coded values and corresponding actual values of the optimization parameters.

Solvent	Code	Extraction temperature (°C)	Extraction time (min)	Number of extraction cycle (times)	Ratio of water to raw material (v/w)
Water	−1	80	50	1	8:1
	0	85	60	2	10:1
	1	90	70	3	12:1

evaluate the antioxidant ability (AOA) of *O. dillenii* crude polysaccharides (OCP).

2. Materials and methods

2.1. Materials and equipments

O. dillenii sample was collected from Charlottetown, Canada. After being ground by grinder machine, powder sample was passed though 20-mesh sieve, and then it was kept in sealed polyethylene bags at −20 °C until used. All solvents and chemicals used were of analytical grade.

2.2. Extraction procedure

In the experiment, extractions of cactus polysaccharide were done in glass bottles using distilled water as solvent. In general (Cordova et al., 2013; Hong et al., 2013; Shen et al., 2013), 10.0 g of *O. dillenii* powder was placed in a glass bottle with 100 ml of solvent, and then placed in a reciprocal shaking water bath at a speed of 100 rpm (2.0 Hz). The solution was filtered through paper filter, and the insoluble residue was treated again as mentioned above. When extraction of polysaccharide was accomplished, the mixture was cooled to room temperature using ice water and centrifuged (6000 rpm, 15 min), the supernatant was concentrated in a rotary evaporator under reduced pressure, and then precipitated by the addition of ethanol (24 h, 4 °C) to a final concentration of 80% (v/v), and the precipitates were collected by centrifugation (6000 rpm, 15 min), washed with pure ethanol, acetone and finally lyophilized to obtain *O. dillenii* crude polysaccharide. The percentage polysaccharides extraction yield (%) is calculated as follows:

$$\text{Polysaccharides extraction yield (\%, w/w)} = \frac{\text{Dried crude polysaccharides weight (g)}}{\text{Powder weight (g)}} \times 100\% \quad (1)$$

2.3. Determination of AOA

DPPH assay was used to evaluate the AOA of crude polysaccharide from *O. dillenii* in the present study. The DPPH free radical scavenging activity of each extract was determined according to the method described by Della (DellaGreca et al., 2009; Pedrini et al., 1993) with slight modification. In briefly, a 0.1 mM of ethanol DPPH solution was prepared. The initial absorbance of the DPPH in ethanol was measured at 517 nm and did not change throughout the period of assay. An aliquot (0.1 ml) of each extract (with appropriate dilution if necessary) was mixed with 3.0 ml of ethanol DPPH solution and incubated for 30 min at 30 °C in dark. Then the absorbance at 517 nm was measured. The DPPH free radical scavenging activity was expressed by IC_{50} value, which was the concentration of extracts, required to decrease the absorbance at 517 nm by 50%. Measurements were performed in triplicate.

2.4. Experimental design

After determining the preliminary range of extraction variables through single-factor test, the response surface methodology (RSM) with the Box–Behnken design (BBD) was employed to determine the optimal combination of extraction variables. As shown in Table 1, the ranges and center point values of four independent variables were based on the results of single factor experiments. The four independent variables chosen for this study were extraction temperature (X_1, °C), extraction time (X_2, min), number of extraction cycle (X_3, times) and ratio of water to raw material (X_4, v/w), while the dependent variable (response variable) was the extraction yield of OCP (%). Each variable was prescribed into three levels, coded +1, 0 and −1 for high, intermediate and low value, respectively. Experimental data was fitted to the following second-order polynomial model:

$$Y = \sum A_0 + \sum_{i=1}^{k} A_i X_i + \sum_{i=1}^{k} A_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} A_{ij} X_i X_j \quad (2)$$

where Y is the response variable, X_i and X_j are the independent variables ($i \neq j$) affecting the response of Y, A_0, A_i, A_{ii} and A_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively.

2.5. Statistical analysis

Statistical analysis of the single-factor experimental data was performed with SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Stat-Ease Design-Expert 8.0.5 (Trial version, Stat-Ease Inc., Minneapolis, MN, USA) was used for the experimental design and the regression analysis of experimental data.

The regression coefficients were determined by analysis of variance (ANOVA). The statistical significance of the regression coefficient was checked by Student's *t*-test, and the second-order model equation was determined by Fischer's *F*-test at a probability (P) of 0.001, 0.01 or 0.05. The adequacy of the model was evaluated by lack of fit, determination coefficient (R^2) and *F*-value obtained from ANOVA.

3. Results and discussion

3.1. Effect of extraction time on yield of OPC

Extraction time, as an important factor, was associated with final concentration of polysaccharides, extraction efficiency and energy cost. In this study, the effect of extraction time on extraction yield of polysaccharides from *O. dillenii* was investigated and the result was shown in Fig. 1. The extraction time was set at 30, 40, 50, 60, 70, 80 and 90 min while other extraction parameters were given as followings: ratio of water to raw material 15:1 and extraction temperature 85 °C. It could be found that the extraction yield increased as extraction time ascended from 30 to 60 min, peaked at 60 min, and then no longer increased when extraction time exceeded 60 min (Fig. 1a).

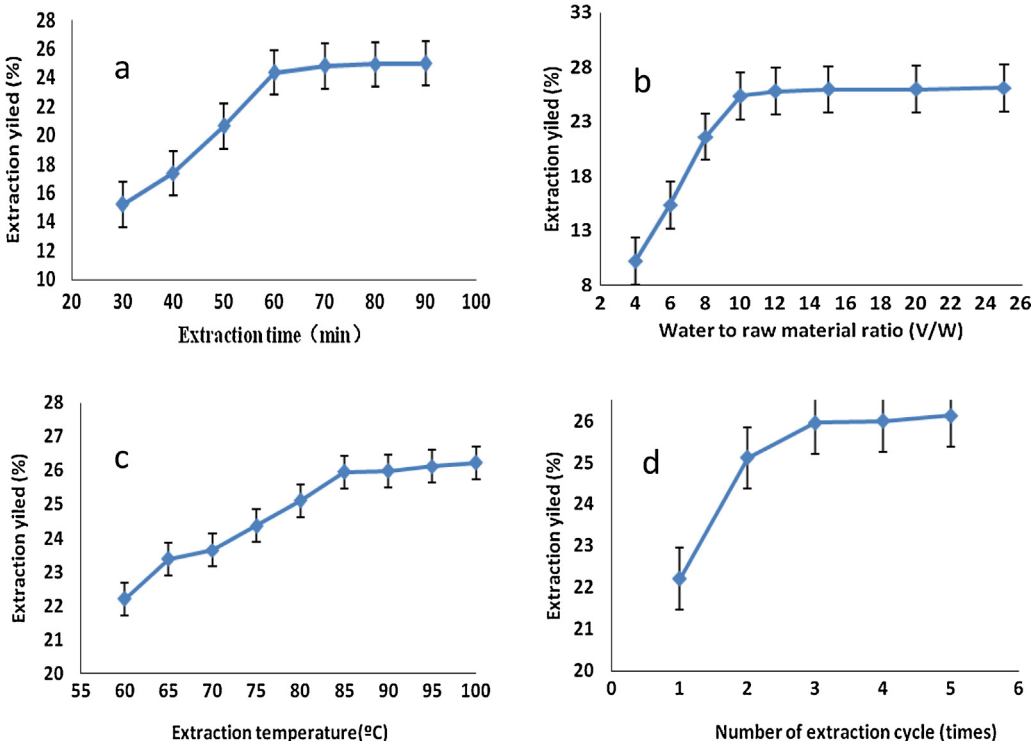

Fig. 1. Effects of different temperature (a), times (b), number of extraction cycle (c) and water to raw material ratio (d) on extraction yield of OCP.

3.2. Effect of ratio of water to raw material on yield of OPC

Ratio of water to raw material, as another important factor, could significantly affect the extraction yield. Too small, polysaccharides in raw material cannot be completely extracted up. Too high, it will cause high process cost. In this study, the effect of ratio of water to raw material on extraction yield of polysaccharides from *O. dillenii* was investigated, and the results were listed in Fig. 1b. The ratio of water to raw material was set at 4:1, 6:1 8:1, 10:1, 12:1, 15:1, 20:1 and 25:1 while other extraction parameters were given as followings: extraction temperature 85 °C and extraction time 60 min. It could be founded that the extraction yield of polysaccharides from *O. dillenii* continued to increase evidently with the increasing ratio of water to raw material. But the growth began to moderate when the ratio of water to raw material exceeded 15 (Fig. 1b).

3.3. Effect of extraction temperature on yield of OPC

To investigate effect of temperature on extraction yield of polysaccharides, a range of temperature from 60 °C to 100 °C has been used for investigation, while the extraction time and ratio of water to raw material were set at 60 min and 15:1, respectively. As shown in Fig. 1c, extraction yield of polysaccharides increased as extraction temperature ascended from 60 °C to 85 °C, peaked at 85 °C, and then no longer increased when the extraction temperature exceeded 85 °C.

3.4. Effect of number of extraction cycles on yield of OPC

In this experiment, the effect of number of extraction cycles on extraction yield of polysaccharides was inspected, the results showed that extraction 2 times has significantly higher extraction yield than extraction one time, and little change occurred when the extraction times was above 2 (Fig. 1d).

3.5. Optimization of extraction parameters of polysaccharides

3.5.1. Statistical analysis and the model fitting

The BBD in the experimental design consisted of four factors, three levels and five replicates at the center point (Table 2), and the five center point runs were carried out to measure the process

Table 2
The coded experimental and predicted for RSM design using water as solvent.

Run	X_1	X_2	X_3	X_4	Extraction yield (%)	
					Experimental	Predicted
1	−1	0	−1	0	13.29	13.32
2	0	−1	−1	0	7.13	7.32
3	−1	0	0	−1	17.78	17.37
4	1	0	−1	0	15.59	15.12
5	0	−1	0	1	19.30	19.06
6	−1	0	1	0	21.94	22.15
7	0	0	1	1	25.30	25.02
8	1	1	0	0	25.24	25.22
9	1	0	0	−1	19.96	19.92
10	0	1	1	0	25.89	25.32
11	0	1	−1	0	16.99	16.64
12	−1	0	0	1	24.27	23.83
13	1	0	1	0	26.02	25.73
14	−1	1	0	0	22.38	22.29
15	0	0	−1	−1	8.84	8.70
16	0	0	0	0	26.66	26.31
17	−1	−1	0	0	14.68	14.25
18	0	1	0	1	27.28	26.97
19	0	−1	0	−1	12.05	12.09
20	1	−1	0	0	17.09	16.70
21	0	0	0	0	26.30	26.31
22	0	0	−1	1	20.00	19.93
23	1	0	0	1	26.75	26.67
24	0	1	0	−1	20.77	20.73
25	0	0	1	−1	23.48	23.05
26	0	0	0	0	26.64	26.31
27	0	0	0	0	26.62	26.31
28	0	0	0	0	26.67	26.31
29	0	−1	1	0	18.12	18.08

Table 3
ANOVA for the effects of extraction temperature (X_1), time (X_2), number of extraction cycle (X_3) and ratio of water to raw material (X_4) on the extraction yield of OCP with water as solvent using predicted polynomial models.

Source	Sum of squares	Df	Mean square	*F*-value	*P*-Value	Significant[a]
Model	935.3424	14	66.8102	842.6110	<0.0001	Significant
A-X_1	22.1809	1	22.1809	279.7457	<0.0001	***
B-X_2	209.9349	1	209.9349	2647.7024	<0.0001	***
C-X_3	289.1636	1	289.1636	3646.9364	<0.0001	***
D-X_4	133.5310	1	133.5310	1684.0946	<0.0001	***
AB	0.0545	1	0.0545	0.6878	0.4208	
AC	0.8046	1	0.8046	10.1478	0.0066	**
AD	0.0225	1	0.0225	0.2843	0.6022	
BC	1.0988	1	1.0988	13.8586	0.0023	**
BD	0.1358	1	0.1358	1.7126	0.2117	
CD	21.8307	1	21.8307	275.3287	<0.0001	***
A^2	32.9645	1	32.9645	415.7486	<0.0001	***
B^2	132.1336	1	132.1336	1666.4713	<0.0001	***
C^2	165.7640	1	165.7640	2090.6188	<0.0001	***
D^2	30.1793	1	30.1793	380.6222	<0.0001	***
Residual	1.1101	14	0.0793			
Lack of fit	1.0142	10	0.1014	4.2307	0.0885	Not significant
Pure error	0.0959	4	0.0240			
Cor total	936.4525	28				
R^2	0.9988					
Adj.R^2	0.9976					
Pred.R^2	0.9936					
Adequate Precision	98.0090					

[a] **Significant at $P<0.01$; ***Significant at $P<0.001$.

stability and inherent variability. The experimental conditions and the results of 29 runs with BBD design were presented in Table 2, and the results were means of triplicate tests. As shown in Table 2, the extraction yield of OCP values (%) varied from 7.13% to 27.28%.

The results of extraction yield affected by extraction temperature, extraction time, number of extraction cycle and the ratio of water to raw material were fitted with a second order polynomial equation, and the values of regression coefficients were calculated.

The effects of four variables were highly significant on extraction yield of OCP (Table 3). The extraction yield value could be expressed by the following second order polynomial equations:

$$Y = 26.58 + 1.36 \times X_1 + 4.18 \times X_2 + 4.91 \times X_3 + 3.34 \times X_4 + 0.45 \times X_1X_3 - 0.52 \times X_2X_3 - 2.34 \times X_3X_4 - 2.25 \times X_1^2 - 4.51 \times X_2^2 - 5.06 \times X_3^2 - 2.16 \times X_4^2 \quad (3)$$

The predicted values of extraction yield based on the above model were shown in Table 2.

The statistical significance of regression equation was checked by *F*-test, and ANOVA for response surface quadratic polynomial model were summarized in Table 3. The results of high model *F*-value (842.6110) and low *P*-value ($P<0.0001$) indicate that the models were highly significant. The determination coefficient (R^2) for model (0.9988) was close to 1.0, which represented the satisfactory correlation between actual and predicted values. The adjusted R^2 (Adj. R^2) value was 0.9976, which meant most variation (>99%) of the extraction yield could be predicted by the model, and less than 1% variations could not be explained by the models.

The lack-of-fit measured the failure of the model to represent the data in the experimental domain at points which are not included in the regression. The *F*-value of 4.2307 and *P*-value of 0.0885 for extraction yield implied that the lack of fit was insignificant relative to the pure error due to noise. Adequate precision compared the range of the predicted values at the design points to the average prediction error. Ratio greater than 4 indicated adequate model discrimination. In this research, the values were well above 4.

The *P*-values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller was the value of *P*, the more significant was the corresponding coefficient. It can be seen from Table 3 that linear coefficients (X_1, X_2, X_3, X_4), quadratic term coefficient (X_1^2, X_2^2, X_3^2, X_4^2) and cross product coefficients ($X_1 \times X_3, X_3 \times X_3, X_3 \times X_4$) were significant with very small *P* values ($P<0.01$). The other term coefficients were not significant ($P>0.05$).

3.5.2. Analysis of response surfaces

The 3D response surface and 2D contour plots are the graphical representations of regression equation. They provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plot indicates that the interactions between the corresponding variables are negligible, while elliptical contour plot indicates that the interactions between the corresponding variables are significant. In this study, the results of extraction yield of OCP affected by extraction temperature, extraction time, number of extraction cycle and water to raw material ratio are presented in Figs. 2 and 3.

Figs. 2a and 3a, which give the extraction yield of OCP as a function of extraction temperature and time at fixed number of extraction cycle (2) and water to raw material ratio (10:1), indicated that the extraction yield increased rapidly with increase of extraction temperature from 80 °C to 85 °C, and increased with the increase of extraction time from 50 min to 65 min. However, with the extraction temperature increased from 85 °C to 90 °C and extraction time from 65 min to 70 min, the extraction yield only has a slight increase.

The extraction yield of OCP affected by different extraction temperature and number of extraction cycle was seen in Figs. 2b and 3b, when the extraction time and water to raw material ratio were fixed at 60 min and 10:1 respectively. Extraction temperature and number of extraction cycle had a positive impact on the extraction yield of OCP. From two figures, we can conclude that the extraction yield of OCP increased with the increase in number of extraction cycle from 1 to 2, and but beyond 2 times, extraction yield of OCP reached the plateau region where the yield was maximized and did not further increase the yield. The extraction yield of OCP was found

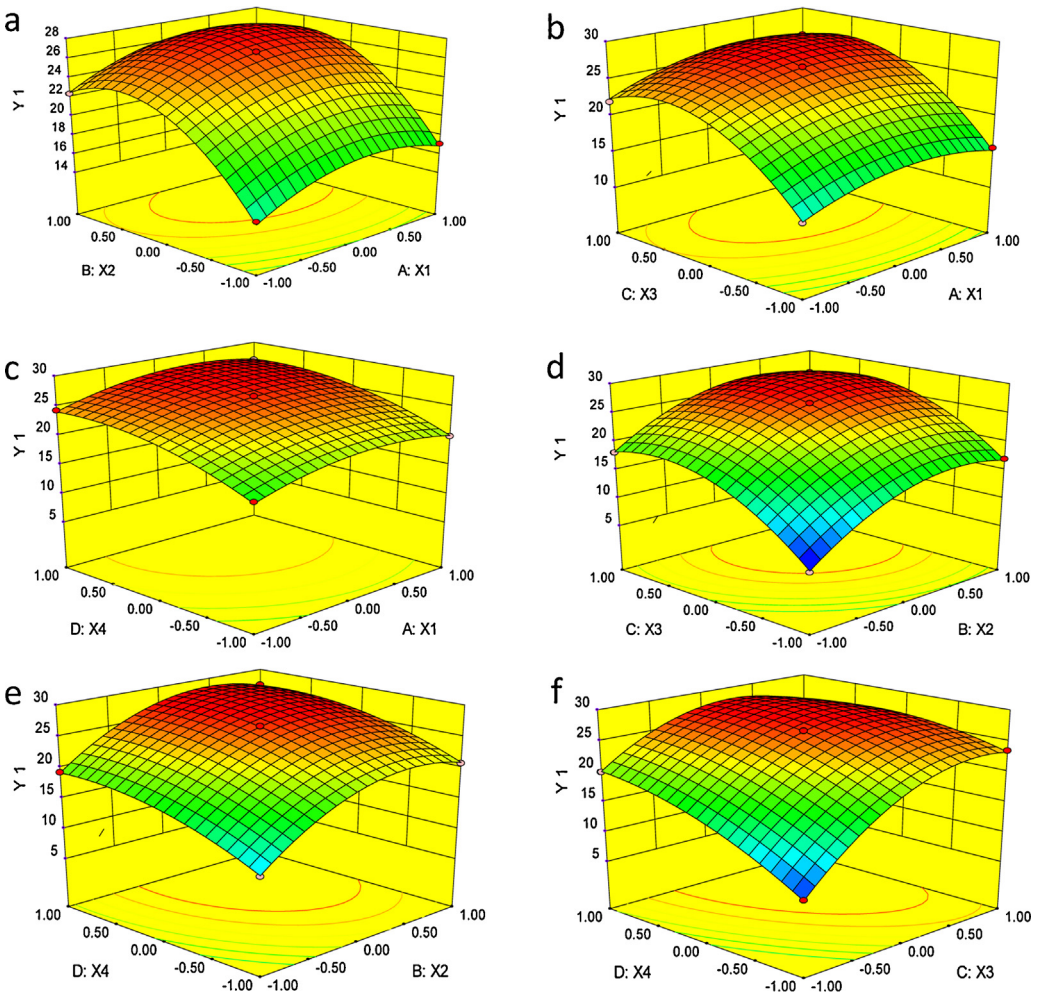

Fig. 2. Response surface (3D) showing the effect of temperature (X_1), times (X_2), number of extraction cycle (X_3) and water to raw material ratio (X_4) on extraction yield of OCP.

to increase rapidly with increase of extraction temperature from 80 °C to 86 °C. It can be seen that the maximum extraction yield of OCP can be achieved when extraction temperature and number of extraction cycle are around 86 °C and 2 times, respectively.

Figs. 2c and 3c show the 3D response surface plot and the contour plot at varying extraction temperature and water to raw material ratio at fixed extraction time and number of extraction cycle. It indicated that the maximum extraction yield of OCP can be achieved when extraction temperature and ratio of water to raw material at the threshold level of around 85 °C and 10:1, respectively.

Figs. 2d and 3d illustrate the 3D response surface plot and the contour plot at varying extraction time and number of extraction cycle at fixed extraction temperature (85 °C) and water to raw material ratio (10:1). The extraction yield of OCP increased with the increasing extraction time from 50 to 70 min and reached the maximum value when the water to raw material ratio at the threshold level of 10:1. So, extraction yield of OCP increased with simultaneously increasing water to raw material ratio and extraction time.

In Figs. 2e and 3e, when the 3D response surface plot and the contour plot were developed for the extraction yield of OCP with varying extraction time and water to raw material ratio at fixed extraction temperature (85 °C) and number of extraction cycle (2 times). The yield increased rapidly with the extraction time. The extraction yield of OCP increased with the increasing ratio of water to raw material from 8:1 to 11:1, and reached the maximum value rapidly at an extraction time around 66 min, but beyond this ratio, extraction yield of OCP did not further increase.

The 3D response surface plot and the contour plot based on the independent variable number of extraction cycle and ratio of water to the raw material were shown in Figs. 2f and 3f, while the other two independent variables, extraction time and temperature were kept at 60 min and 85 °C respectively. An increase in the extraction yield of OCP could be significantly achieved with the increasing of extraction number. It was observed that the extraction yield of OCP increased with the ratio of water to raw material from 8:1 to 11:1, meaning that further increase of water to raw material ratio would not increase the extraction yield of OCP any longer.

3.6. Verification of predictive model

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. In order to validate the adequacy of the model equations, verification experiment was carried out under the optimal conditions: extraction temperature 85 °C, extraction time 63.7 min, extraction 2 times and ratio of water to raw material 11.14:1. Good agreement must exist between the values predicted using model equations and the experimental values at the points of interest. To ensure the predicted result was not biased toward the practical value, experimental rechecking was performed using this deduced optimal condition. This set of conditions was determined to be optimal by the RSM optimization approach and was

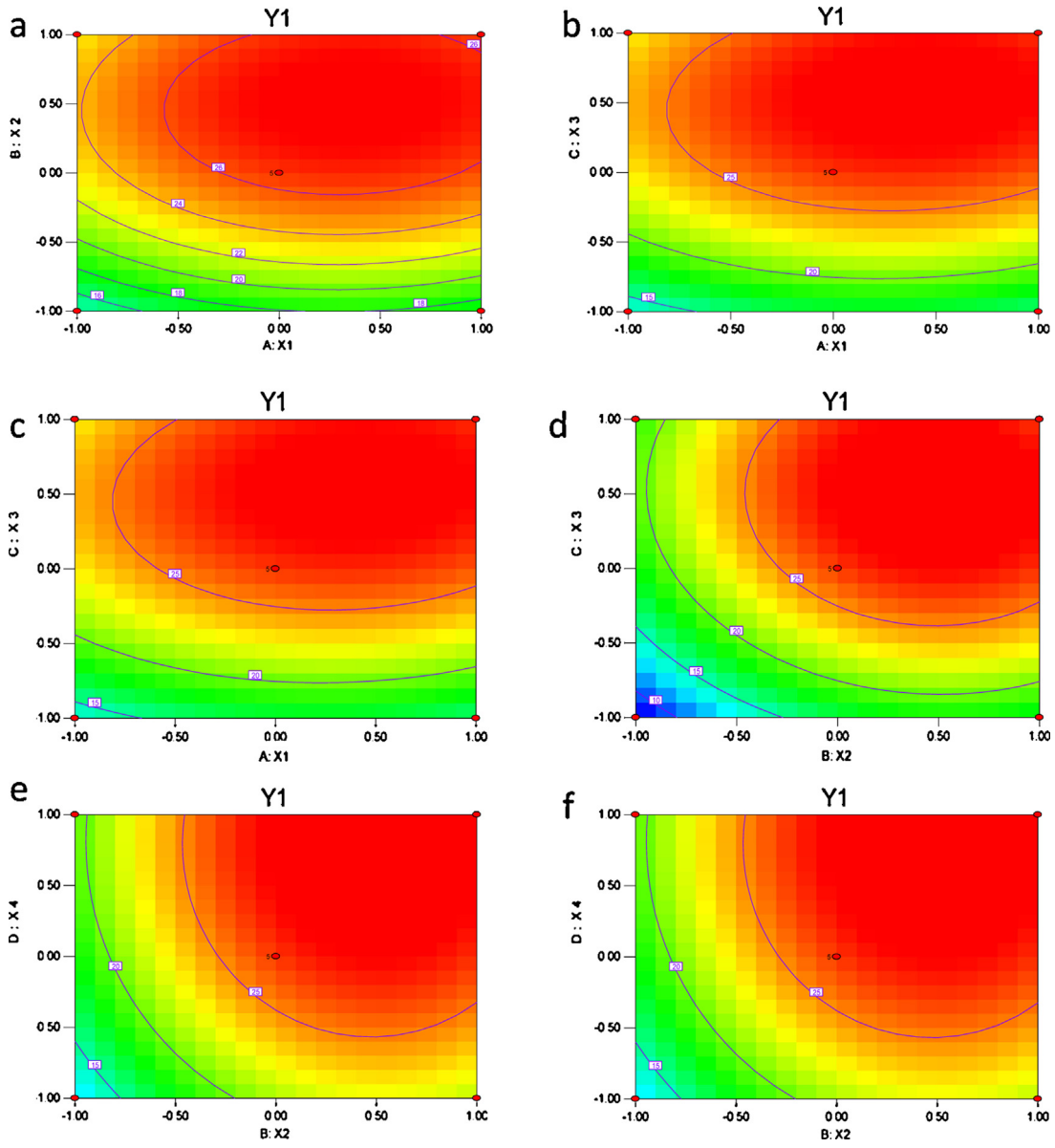

Fig. 3. Contour plots showing the effect of temperature (X_1), times (X_2), number of extraction cycle (X_3) and water to raw material ratio (X_4) on extraction yield of OCP.

also used to validate experimentally and predict the values of the response using the model equation. A mean value of 27.36 ± 0.22% (n = 5), obtained from real experiments, demonstrated the validation of RSM model. The validation result revealed that there was no significant difference between experimental and predicted values, suggesting that the response model was adequate for reflecting the expected optimization (Table 4). This result of analysis indicated that the experimental values were good agreement with the predicted ones, and also suggested that the model of Eq. (3) is satisfactory and accurate.

Table 4
Result of model validation experiments.

No.	Optimum conditions				Extraction yield of OCP (%)	
	Temperature (°C)	Time (min)	Number of extraction cycle (times)	Ratio of water to raw material (v/w)	Experimental	Predicted
1	85	64	2	11:1	27.33	27.44
2	85	64	2	11:1	27.39	27.44
3	85	64	2	11:1	27.34	27.44
4	85	64	2	11:1	27.36	27.44
5	85	64	2	11:1	27.38	27.44
Average					27.36	

Table 5
Results of antioxidant ability for OCP ($n = 3$).

No.	OCP extract purity (%)	IC_{50} ($\mu g\,ml^{-1}$)
1	27.36	3105.53 ± 1.21
2	33.22	2756.62 ± 1.14
3	39.68	2355.85 ± 1.03
4	43.66	2102.54 ± 1.36
5	47.51	1889.35 ± 1.25
6	60.22	1236.62 ± 1.18
7	70.37	1008.37 ± 1.32
8	75.88	856.34 ± 1.20
9	83.15	607.69 ± 1.23
10	90.12	408.32 ± 1.03

3.7. Analysis of AOA for OCP

DPPH assay was used to evaluate the AOA of different purity extract of OCP (purity from 27.36% to 90.12%) from *O. dillenii* in the present study. As shown in Table 5, with the purity of OCP increasing, the IC_{50} for scavenging activity on DPPH free radical was decreasing, from $3105.53 \pm 1.21\ \mu g\,ml^{-1}$ to $408.32 \pm 1.03\ \mu g\,ml^{-1}$, which revealed that OCP has good antioxidant ability.

4. Conclusion

RSM was used to optimize the optimum condition for extraction of *O. dillenii* crude polysaccharide. The coefficients of determination (0.9988) and the probability value ($P < 0.0001$) for the regression model demonstrate a very high significance for predicting the responses. In the case of water as solvent, optimal extraction conditions for *O. dillenii* crude polysaccharide are obtained as follows: extraction temperature 85 °C, extraction time 63.7 min, extraction 2 times and ratio of water to raw material 11.14:1. Under this condition, the mean experimental value of extraction yield ($27.36 \pm 0.22\%$) was achieved, which corresponds well with the predicted values. In addition, the results of DPPH experiment suggest that the *O. dillenii* crude polysaccharide has significant antioxidant ability.

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