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Optimization of extraction process of crude polysaccharides from *Plantago asiatica* L. by response surface methodology

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ABSTRACT

Polysaccharide production from the aerial parts of *Plantaoo asiatica* L. was carried out using water decoction. Response surface methodology (RSM), based on a five level, four variable central composite design (CCD), was employed to obtain the best possible combination of extraction time (X_1: 1–5 h), extraction temperature (X_2: 60–100 °C), number of extraction (X_3: 1–5), and ratio of water to raw material (X_4: 10–30) for maximum polysaccharide production. The experimental data obtained were fitted to a second-order polynomial equation using multiple regression analysis and also analyzed by appropriate statistical methods (ANOVA). The optimum extraction conditions were as follows: extraction time of 3.9 h, extraction temperature of 91 °C, number of extraction of 4, and ratio of water to raw material of 24. Under these conditions, the experimental yield was 4.37 ± 0.13%, which is well in close agreement with value predicted by the model.

1. Introduction

The aerial parts of *Plantaoo asiatica* L., 'Plantago Herba', have been used since ancient times as a diuretic, an antiinflammatory and an anti-asthmatic drug, in China and Japan (Nishibe, Tamayama, Sasahara, & Andary, 1995; Ramkumar & Rao, 2005). This herbal medicine has also been shown to possess antileukemia, anticarcinoma and antiviral activities, as well as activities which modulate cell-mediated immunity (Chiang, Chiang, Chang, & Lin, 2003). In the past several years, medicinal plant polysaccharides have been widely studied for their chemical properties and biological activities (Guo, Zou, & Sun, 2010; Liang, 2008; Schepetkin & Quinn, 2006; Sun, Liu, & Kennedy, 2010; Zhong & Wang, 2010). Whereas, little attention was devoted to the extraction of the aerial parts of *P. asiatica* polysaccharides (PAP). Therefore, we reported the optimization of extracting parameters for the production of PAP.

Response surface methodology (RSM) is an effective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Giovanni, 1983). It is widely used in optimizing the extraction process variables, such as polysaccharides, anthocyanins, vitamin E, phenolic compounds and protein from varied materials (Gan, Abdul Manaf, & Latiff, 2010a; Gan, Abdul Manaf, & Latiff, 2010b; Gan & Latiff, in press; Ge, Ni, Yan, Chen, & Cai, 2002; Li & Fu, 2005; Qiao et al., 2009).

The purpose of the present study was to optimize the process for production of polysaccharides from the aerial of *P. asiatica*, using response surface methodology (RSM), employing a CCD (4 factors and 5 levels) to study the effects of extraction time, extraction temperature, number of extraction, and ratio of water to raw material on the extraction yield of PAP.

2. Materials and methods

2.1. Materials

The aerial parts of *P. asiatica* L. were purchased from Bozhou, Anhui Province, China. All other chemicals were of analytical grade.

2.2. Extraction of crude polysaccharides

Dried aerial parts of *P. asiatica* L. (30.0 g) were ground in a high speed disintegrator (Model SF-2000, Chinese Traditional Medicine Machine Works, Shanghai, China) to obtain a fine powder, then were extracted in a Soxhlet apparatus with aether (20–40 °C), and pretreated with 80% aether twice to remove some colored materials, oligosaccharides and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained, as described previously (Yang, Qu, & Cheng, 2004; Zykwinska, Rondeau-Mouro, Garnier, Thibault, & Ralet, 2006). The pretreated dry powder (30.0 g) was extracted with deionized water

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Table 1
Independent variables and their levels used in the response surface design.

Independent variables	Factor level				
	−2	−1	0	1	2
Extraction time (h)	1	2	3	4	5
Extraction temperature (°C)	60	70	80	90	100
Number of extraction	1	2	3	4	5
Ratio of water to raw material	10	15	20	25	30

(water–material (ml/g) ranging from 5:1 to 35:1) at pH 6.5–7.5 (adjusting the suspension pH by 0.1 mol/L NaOH or HCl), while the temperature of the water bath was kept steady for a given temperature (within ±1.0 °C, extraction temperature ranging from 40 to 100 °C). The water–material slurry in a 2.0 L stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 1 to 7 h) during the entire extraction process. The extracted slurry was centrifuged at 4200 rpm/min for 20 min to collect the supernatant, and the insoluble residue was treated again (extraction times ranging from 1 to 7) as mentioned above.

The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (Senco Technology and Science Inc., Shanghai, China) at 55 °C under vacuum. The resulting solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept overnight at 4 °C. Then the solution was centrifuged at 4200 rpm/min for 20 min, washed six times with dehydrated ethanol, and the precipitate was collected as crude extract. The extract was air-dried at 50 °C until its weight was constant, and then was weighted with a balance (BS2202S, SARTORIUS, Germany). The percentage polysaccharides yield (%) is calculated as follows:

$$\text{polysaccharides yield}\,\%\,(\text{w/w}) = \frac{\text{dried crude extraction weight}}{\text{powder weight (30 g)}} \times 100\%$$

2.3. Experimental design and statistical analysis

After determining the preliminary range of the extraction variables through single-factor test, a central composite design (CCD) with four independent variables (X_1, extraction time; X_2, extraction temperature; X_3, number of extraction; X_4, ratio of water to raw material) at five levels was performed. For statistical calculation, the variables were coded according to

$$x_i = \frac{X_i - X_0}{\Delta X_i} \tag{1}$$

where x_i is the independent variable coded value, X_i is the independent variable real value, X_0 is the independent variable real value on the centre point and ΔX_i is the step change value. The range of independent variables and their levels is presented in Table 1. The independent variables and their ranges were chosen based on preliminary experiment results.

The whole design consisted of 31 experimental points carried out in random order, which included 16 factorial points, 7 centre points and 8 axial points at the centre of the design was used for the estimation of a pure error sum of squares.

Data from CCD were analyzed by multiple regressions to fit the following quadratic polynomial model.

$$Y = \beta_{k0} + \sum_{i=1}^{4} \beta_k x_i + \sum_{i=1}^{4} \beta_k x_i^2 + \sum_{i\langle j=2}^{4} \beta_k x_i x_j \tag{2}$$

Y represent the response function. β_{k0} is an intercept. Where β_{ki}, β_{kii} andβ_{kij} are the coefficients of the linear, quadratic and interactive terms, respectively. And accordingly x_i and x_j represent the coded independent variables, respectively. The fitted polynomial equation is expressed as surface and contour plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions (Lu, Engelmann, Lila, & Erdman, 2008). The analysis of variance tables was generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The regression coefficients were then used to make statistical calculation to generate dimensional and contour maps from the regression models. Design-Expert 7.1.6 (Trial Version, State-Ease Inc., Minneapolis, MN, USA) software package was used to analyze the experimental data. P-values of less than 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Effect of different times on extraction yield of PAP

Extraction time is a factor that would influence the extraction efficiency and selectivity of the fluid. This might be due to the time requirement of the exposure of the PAP to the release medium where the liquid penetrated into the dried powdered material, dissolved the PAP and subsequently diffused out from the material. A longer extraction time presents a positive effect on the yield of polysaccharides. It was reported that a long extraction time favors the production of polysaccharides (Hou & Chen, 2008; Liu, Wei, Guo, & Kennedy, 2006Liu et al., Wei et al., 2006). The effect of different times on extraction yield of PAP is shown in Fig. 1a. Extraction was carried out at different time conditions while other extraction variables were set as follows: extraction temperature of 80 °C, number of extraction of 3, and ratio of water to raw material of 20. When extraction time varied from 1 to 3 h, the variance of extraction yield was relatively rapid, and polysaccharide production reached a maximum at 3–4 h, and then no longer changed as the extraction proceeded. This indicated that extraction time of 3 h was sufficient to obtain the polysaccharide production. Thus, extraction of 3–4 h was favorable for producing the polysaccharides.

3.2. Effect of different temperatures on extraction yield of PAP

The increase of the polysaccharides diffusion coefficient and the enhanced solubility of the polysaccharides in the extracting solvent at higher temperatures caused the increase of the polysaccharides mass going out from the mushroom particles into the solution (Li, Cui, & Kakuda, 2006). The extraction coefficient increased with increasing the extraction temperature due to the increase of the polysaccharides solubility (Braga, Moreschi, & Meireles, 2006). To study the effect of different temperatures on extraction yield of PAP, extraction process was carried out using the different extraction temperatures of 40, 50, 60, 70, 80, 90 and 100 °C. The extraction time, number of extraction and ratio of water to raw material were fixed at 2 h, 3 and 20, respectively. The extraction yield of PAP had been increasing when extraction temperature increased from 40 to

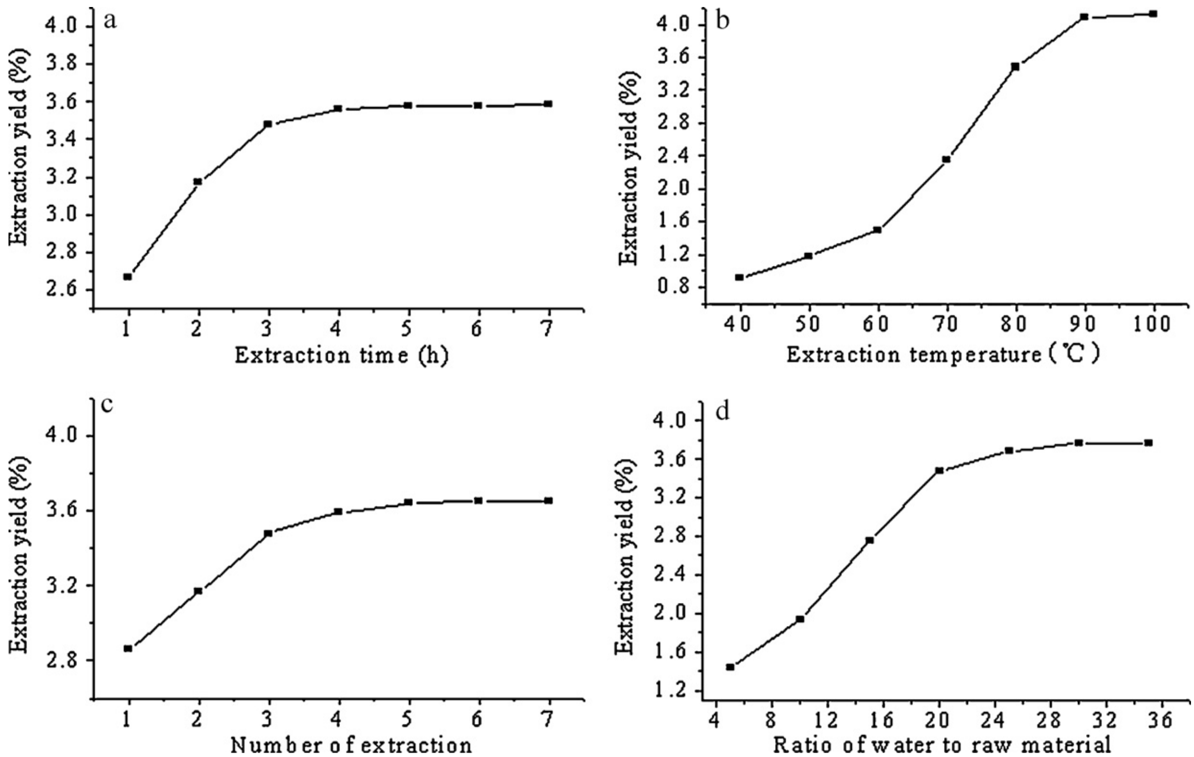

Fig. 1. Effects of different (a) times, (b) temperatures, (c) numbers of extraction, and (d) ratios of water to raw material on extraction yield of PAP.

90 °C. As shown in Fig. 1b, the maximum yield of PAP was observed when extraction temperature was 90 °C. This tendence is in agreement with reports of other authors in extracting polysaccharides (Vinogradov, Brade, Brade, & Holst, 2003). Although the extraction yield of polysaccharides was also high at 100 °C, increasing temperature will bring about the increase in cost for the extraction process from the industrialisation point of view. Therefore, extraction temperature range of 90–100 °C was considered to be optimal in the present experiment.

3.3. Effect of number of extraction on extraction yield of PAP

The effect of number of extraction on extraction yield of PAP is shown in Fig. 1c. Extraction was carried out at different numbers of extraction (1–7) conditions when other extraction condition was as following: extraction temperature of 80 °C, extraction time of 3 h, and ratio of water to raw material of 20. The extraction yields of the polysaccharides significantly increased from 2.86% to 3.64% as number of extraction increased from 1 to 5. However, when number of extraction continued to increase, the extraction yields no longer changed. The highest extraction yield was observed when the number of extraction was 5. This tendence is in agreement with reports of other authors in extracting polysaccharides (Liang, 2008; Sun et al., 2010).

3.4. Effect of different ratios of water to raw material on extraction yield of PAP

The effect of different ratios of water to raw material on extraction yield of PAP is shown in Fig. 1d. Extraction was carried out at different ratios of water to mushroom (5–35) conditions while other extraction parameters were as follows: extraction temperature of 80 °C, extraction time of 3 h, and number of extraction of 3. The extraction yields of the polysaccharides significantly increased from 1.43% to 3.77% as the ratio of water to raw material increased from 5 to 30 shown in Fig. 1d, due to the increase of the driving force for the mass transfer of the polysaccharides (Bendahou, Dufresne, Kaddami, & Habibi, 2007). However, when the ratio continued to increase, the extraction yields no longer changed.

According to the single-parameter study, we adopted extraction time of 1–5 h, extraction temperature of 60–100 °C, number of extraction of 1–5, and ratio of water to raw material of 10–30 for RSM experiments.

3.5. Statistical analysis and the model fitting

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. There were a total of 31 runs for optimizing the four individual parameters in the current CCD. Table 2 shows the experimental conditions and the results of extraction yield of PAP according to the factorial design. Maximum extraction yield of PAP (4.24%) was recorded under the experimental conditions of extraction time of 4 h, extraction temperature of 90 °C, number of extraction of 4, and ratio of water to raw material of 25. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by the following second-order polynomial equation:

$$
\begin{aligned}
Y = {} & 3.48 + 0.275 \times X_1 + 0.69667 \\
& \times X_2 + 0.29333 \times X_3 + 0.52667 \times X_4 + 0.04875 \\
& \times X_1 \times X_2 - 0.01 \times X_1 \times X_3 + 0.0575 \times X_1 \times X_4 \\
& + 0.0175 \times X_2 \times X_3 - 0.2075 \times X_2 \times X_4 + 0.03875 \times X_4 \\
& - 0.16104 \times X_1 \times X_1 - 0.24104 \times X_2 \times X_3 \times X_3 \\
& - 0.22979 \times X_4 \times X_4
\end{aligned}
\tag{3}
$$

The fit statistics of extraction yield (Y_1) for the selected quadratic predictive model is shown in Table 3. The coefficient of the variation (CV) and value of adjusted determination coefficient ${R_{adj}}^2$

Table 2
Response surface central composite design (uncoded) and results for extraction yield of PAP.

No.	X_1/extraction time (h)	X_2/extraction temperature (°C)	X_3/number of extraction	X_4/ratio of water to raw material	Extraction yield (%)
1	−1	−1	−1	−1	0.68
2	−1	−1	−1	1	1.76
3	−1	−1	1	−1	0.93
4	−1	−1	1	1	2.67
5	−1	1	−1	−1	2.44
6	−1	1	−1	1	2.89
7	−1	1	1	−1	3.04
8	−1	1	1	1	3.79
9	1	−1	−1	−1	0.98
10	1	−1	−1	1	2.71
11	1	−1	1	−1	1.77
12	1	−1	1	1	3.36
13	1	1	−1	−1	2.74
14	1	1	−1	1	3.65
15	1	1	1	−1	3.53
16	1	1	1	1	4.24
17	−2	0	0	0	2.67
18	2	0	0	0	3.58
19	0	−2	0	0	1.49
20	0	2	0	0	4.12
21	0	0	−2	0	2.86
22	0	0	2	0	3.64
23	0	0	0	−2	1.93
24	0	0	0	2	3.77
25	0	0	0	0	3.48
26	0	0	0	0	3.48
27	0	0	0	0	3.48
28	0	0	0	0	3.48
29	0	0	0	0	3.48
30	0	0	0	0	3.48
31	0	0	0	0	3.48

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

Source	SS	DF	MS	F-value	Prob > F
Model	26.42	14	1.89	22.35	<0.0001
Residual	1.35	16	0.084		
Lack of fit	1.35	10	0.14		
Pure error	0.000	6	0.000		
Cor total	27.77	30			
	$R^2 = 0.9513$	$R_{adj}^2 = 0.9088$	CV = 10.05		

were 10.05 and 0.9088, respectively, which indicated a high degree of precision of reliability of the experimental values and a high degree of correlation between the observed and predicted values. The ANOVA analysis was shown in Table 4. The P-values were used as a tool to check the significance of each coefficient. The smaller the P-value was, the more significant the corresponding coefficient was (Guo et al., 2010). It can be seen from this table that the linear coefficients (X_1, X_2, X_3, X_4), a quadratic term coefficient ($X_1{}^2$, $X_2{}^2$, $X_3{}^2$, $X_4{}^2$) and cross product coefficients ($X_2 \times X_4$) were significant, with very small P-values ($P < 0.05$). The other term coefficients were not significant ($P > 0.05$). The full model filled Eq. (3) was made three-dimensional and contour plots to predict the relationships between the independent variables and the dependent variables.

3.6. Optimization of extraction conditions of PAP

The graphical representations of the regression Eq. (3), called the response surfaces and the contour plots were obtained using

Table 4
Estimated regression model of relationship between response variables (yield of PAP) and independent variables (X_1, X_2, X_3, X_4).

Variables	DF	SS	MS	F-value	P-value
X_1	1	1.81	1.81	21.5	<0.001
X_2	1	11.65	11.65	137.96	0.003
X_3	1	2.07	2.07	24.46	<0.001
X_4	1	6.66	6.66	78.84	0.001
$X_1 \times X_1$	1	0.74	0.74	8.78	0.0091
$X_1 \times X_2$	1	0.038	0.038	0.45	0.5117
$X_1 \times X_3$	1	1.6×10^{-3}	1.6×10^{-3}	0.019	0.8922
$X_1 \times X_4$	1	0.053	0.053	0.63	0.4402
$X_2 \times X_2$	1	1.66	1.66	19.68	0.0004
$X_2 \times X_3$	1	4.9×10^{-3}	4.9×10^{-3}	0.058	0.8127
$X_2 \times X_4$	1	0.69	0.69	8.16	0.0114
$X_3 \times X_3$	1	0.48	0.48	5.71	0.0296
$X_3 \times X_4$	1	0.024	0.024	0.28	0.6011
$X_4 \times X_4$	1	1.51	1.51	17.88	0.0006

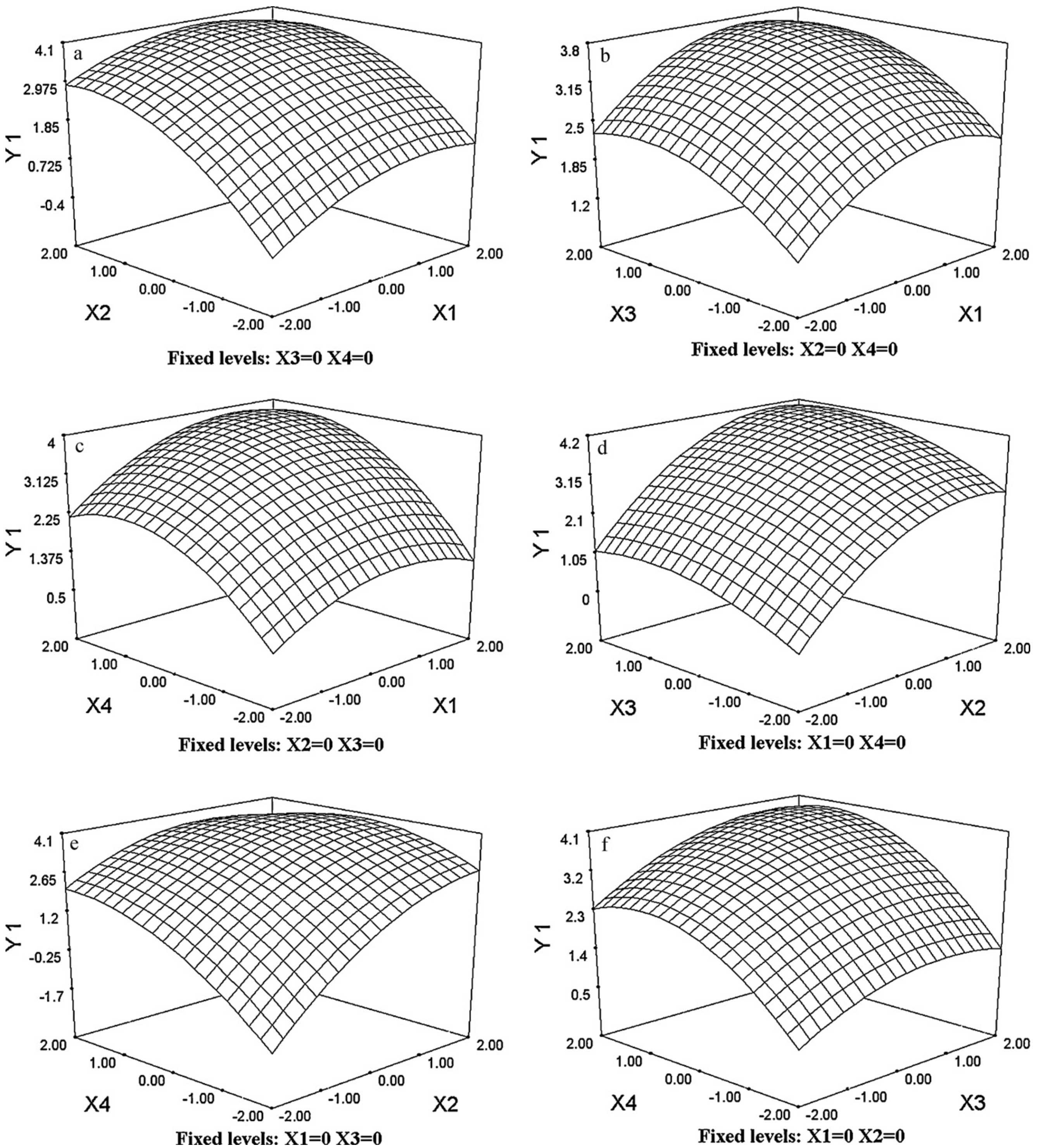

Fig. 2. Response surface (3-D) showing the effect of the extraction time, extraction temperature, number of extraction and ratio of water to raw material on the response Y_1.

Design-Expert, and the results of extraction yield of PAP affected by extraction time, extraction temperature, ratio of water to raw material and number of extraction are presented in Figs. 2 and 3. Response surface methodology plays a key role in identifying the optimum values of the independent variables efficiently, under which dependent variable could arrive the maximum response. In the response surface plot and contour plot, the extraction yield of PAP was obtained along with two continuous variables, while the other two variables were fixed constant at their respective zero level (centre value of the testing ranges). 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 (Muralidhar, Chirumamila, Marchant, & Nigam, 2001). The independent variables and maximum predicted values from the figures corresponded with the optimum values of the dependent variables (responses) obtained by the equations.

The 3-D response surface plot and the contour plot in Figs. 2 and 3a, which give the extraction yield of PAP as a function of extraction time and temperature at fixed number of extraction

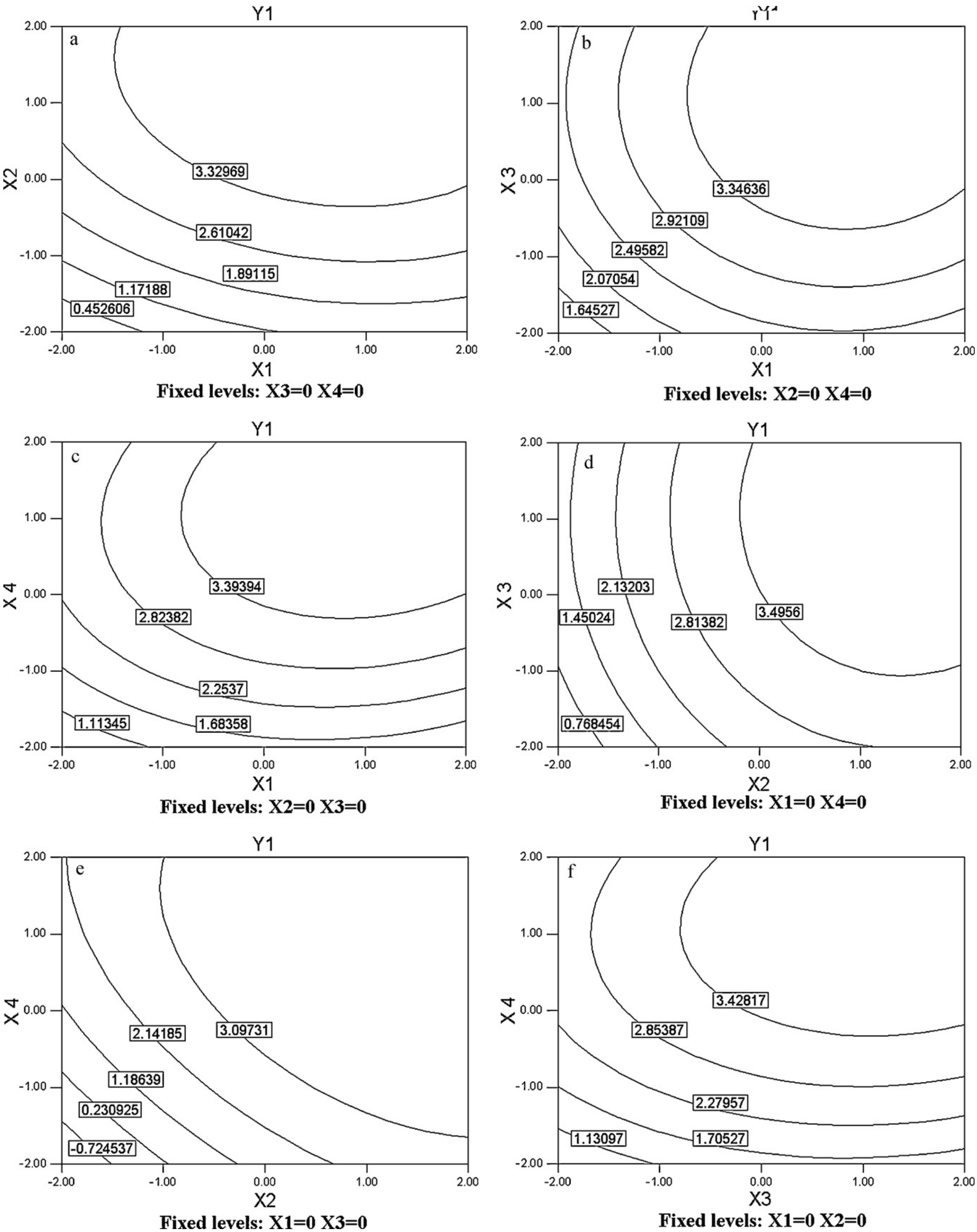

Fig. 3. Contour plots showing the effect of the extraction time, extraction temperature, number of extraction and ratio of water to raw material on the response Y_1.

Table 5
Predicted and experimental values of the responses at optimum conditions.

Optimum condition				Extraction yield of PAP (%)	
Extraction time	Extraction temperature	Number of extraction	Ratio of water to raw material	Experimental[a]	Predicted
3.9 h	91 °C	4	24	4.37 ± 0.13	4.39

[a] Mean ± standard deviation (n = 3).

(0 level) and ratio of water to raw material (0 level), indicated that the extraction yield of PAP increased with increase of extraction time from 1 to 3.9 h, then decreased slightly from 3.9 to 5 h, and increased rapidly with increase of extraction temperature from 85 to 91 °C, then dropped from 91 to 100 °C. Figs. 2 and 3b shows the 3-D response surface plot and the contour plot at varying extraction time and number of extraction at fixed extraction temperature (0 level) and ratio of water to raw material (0 level). From two figures, we can concluded that the extraction yield of PAP increased with increase of number of extraction from 1 to 4, then decreased from 4 to 5, and extraction yield of PAP was found to increase rapidly with increase of extraction time from 1 to 3.9 h, but beyond 3.9 h, extraction yield of PAP reached the plateau region where the yield was maximized and did not further increase the yield. Figs. 2 and 3c shows the 3-D response surface plot and the contour plot at varying extraction time and ratio of water to raw material at fixed extraction temperature (0 level) and number of extraction (0 level). It indicated that the maximum extraction yield of PAP can be achieved when extraction time and ratio of water to raw material at the threshold level of 3.9 h and 24, respectively. The extraction yield of PAP affected by different extraction temperatures and numbers of extraction was seen in Figs. 2 and 3d, when other two variables (extraction time and ratio of water to raw material) were fixed at 0 level. It can be seen that the extraction yield of PAP increased with increase of number of extraction from 1 to 4, then did not further increase with increasing number of extraction, and reached the maximum value when extraction temperature at 91 °C, and beyond this level, extraction yield of PAP did not further increase. In Figs. 2 and 3e, when the 3-D response surface plot and the contour plot were developed for the extraction yield of PAP with varying extraction temperature and ratio of water to raw material at fixed extraction time (0 level) and number of extraction (0 level). It indicated that the maximum extraction yield of PAP can be achieved when extraction temperature and ratio of water to raw material at the threshold level of 91 °C and 24, respectively. The 3-D response surface plot and the contour plot based on independent variables number of extraction and ratio of water to raw material are shown in Figs. 2 and 3f, while the other two independent variables, extraction temperature and extraction time were kept at a zero level. It indicated that the maximum extraction yield of polysaccharides can be achieved when number of extraction and ratio of water to raw material is at the threshold level of 4 and 24, respectively.

From Figs 2 and 3, it can be concluded that optimal extraction condition of polysaccharides from *P. asiatica* L. is extraction time of 3.9 h, extraction temperature of 91 °C, number of extraction of 4, and ratio of water to raw material of 24. Among the four extraction parameters studied, extraction temperature was the most significant factor to affect the extraction yield of PAP, followed by the ratio of water to raw material, number of extraction and extraction time according to according to gradient of slope in the 3-D response surface plot (Fig. 2).

3.7. Verification of predictive model

The suitability of the model equations for predicting optimum response values was tested under the conditions: extraction time of 3.9 h, extraction temperature of 91 °C, number of extraction of 4, and ratio of water to raw material of 24. This set of conditions was determined to be optimum by the RSM optimization approach and was also used to validate experimentally and predict the values of the responses using the model equation. A mean value of 4.37 ± 0.13% (n = 3), obtained from real experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process (Table 5).

4. Conclusion

The extraction conditions have significant effects on the purity of CPP. Using the contour and surface plots in RSM was effective for estimating the effect of four independent variables (extraction time, h; extraction temperature, °C; number of extraction and ratio of water to raw material). The optimum set of the independent variables was obtained graphically in order to obtain the desired levels of crude polysaccharides extraction. The optimal experimental extraction yield of 4.37 ± 0.13% was obtained when the optimum conditions of polysaccharides extraction were the extraction time of 3.9 h, extraction temperature of 91 °C, number of extraction of 4, and ratio of water to raw material of 24. Under these optimized conditions the experimental purity of polysaccharides agreed closely with the predicted yield of 4.39%.

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