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Dodonaea viscosa var. angustifolia leaf: New source of polysaccharide and its anti-oxidant activity

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ABSTRACT

Ultrasonic technology was applied for polysaccharide extraction from the leaves of *Dodonaea viscosa* and response surface methodology (RSM) was used to optimize the effects of processing parameters on polysaccharide extraction yield. Three independent variables were extraction time (X_1), extraction temperature (X_2) and ultrasonic power (X_3), respectively. The statistical analysis indicated the independent variables (X_1, X_2, X_3), the quadratic terms (X_{11} and X_{33}) and the interaction terms (X_1X_2, X_1X_3, X_2X_3) had significant effects on the yield of polysaccharides ($P<0.05$). The optimal extraction conditions of *D. viscosa* leaf were determined as follows: extraction time 50.54 min, extraction temperature 85 °C and ultrasonic power 400 W. Under these conditions, the experimental yield of polysaccharides was $9.455 \pm 0.24\%$, which was agreed closely with the predicted value (9.398%). The evaluation of anti-oxidant activity suggested that the polysaccharide exhibited significant protection against DPPH and hydroxyl radicals and could be explored as a nutraceutical agent.

1. Introduction

Ultrasonic-assisted extraction is one of the important techniques for extracting the valuable compounds from the plant materials (Vilkhu, Mawsonm, Simons, & Bates, 2008). Compared with other extraction techniques such as supercritical fluid extraction and microwave-assisted extraction, the ultrasonic extraction is cheaper and its operation is much easier (Chen et al., 2008; Wang, Cheng, Mao, Fan, & Wu, 2009). The enhanced extraction by ultrasonic treatment is mainly attributed to its mechanical effects, which greatly facilitate mass transfer between immiscible phases through a super agitation, especially at low frequency (Vinatoru et al., 1997).

Dodonaea viscosa is a stiff bushy plant. Muthuvan tribes and Tamilian native who reside in the Shola forest regions of Kerala use leaves of this plant for headaches and backaches. It is commonly known as 'virali'. Water boiled with leaves is used to foment swellings, backaches and used for steam inhalation in cough and colds (Kishore & Sasidharan, 2002). *D. viscosa* is used for stomach pain and piles. It is also used to heal simple ulcer (Perry & Metzger, 1980). *D. viscosa* has been reported to have anti-inflammatory and antimicrobial activity (Getie et al., 2003; Khalil, Sperotto, & Manfron, 2006). It has been also reported to have local anesthetic and smooth muscle relaxant activity (Rojas, Cruz, Ponce-Monter, & Mata, 1996).

Extraction of polysaccharides is an important processing for its application or further research and development, which has prompted numerous research papers on the extraction technology of polysaccharides from plentiful of plants or fungus in recent years (Chen et al., 2010).

The formation of some diseases, such as cancer, can be directly induced by free radicals, while the radical scavenging activity is one of the important functional properties for bioactive compounds (Athukorala et al., 2006; Yang, Zhao, Shi, & Yang, 2008). The radical scavenging activity is often used to evaluate the capacity of antioxidant compounds (Prior & Cao, 1999). Recent studies demonstrated that the antioxidant activity of polysaccharides was related to their degree of polymerization and structure (Chen & Yan, 2005).

Response surface methodology (RSM) is a collection of mathematical and empirical techniques useful for establishing models, and for optimizing processes even in the presence of complex interactions (Mirhosseini, Tan, Hamid, & Yusof, 2008a, 2008b). This procedure not only determines the interaction between parameters, but also reduces the number of experimental trials, development time and overall cost (Myers & Montgomery, 2002). However, there is no information about the extraction and antioxidant activity of polysaccharides from *Myrtle communis* leaf.

The aim of the present study is to extract the polysaccharide from *D. viscosa var. angustifolia* leaf using ultrasonic method and

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evaluate its antioxidant properties. Such information is valuable as plant extracts containing high antioxidant activities can prove beneficial for maintenance of optimal health and may increase the demand of these bioactive substances by food, cosmetic and pharmaceutical industries.

2. Materials and methods

2.1. Materials

The *D. viscosa* leaves were collected from a Mollasani, Khouzestan province, thoroughly washed with distilled water, freeze-dried and ground. All other chemicals and solvents used were of analytical grade and obtained from Merck Co., Germany.

2.2. Methods

2.2.1. Ultrasonic extraction and determination of polysaccharides

The extraction of polysaccharides from *D. viscosa* leaf (DVP) was performed using a method described by Pan et al. (2010).

Ground *D. viscosa* leaf was refluxed with 95% ethanol at 60 °C in a water bath for 3 h to deactivate the endogenous enzymes and remove some soluble materials, including free sugars, amino acids and some phenols. The ethanolic mixture extract was centrifuged (4000 × *g*, 10 min). The ethanol extraction was washed twice with 95% ethanol. The combined extract was vacuum-dried at 50 °C for 12 h, and it was suspended in the water and sonicated with ultrasonic bath at the temperature of 50–100 °C and ultrasound power of 100–700 W for 20–100 min. The supernatant was concentrated in a rotary evaporator under reduced pressure at 55 °C, and then mixed with 4-fold volumes of ethanol 95% and kept at 4 °C for 24 h. After centrifugation at 5000 rpm/min for 15 min, the precipitate was washed three times with anhydrous ethanol and then dialyzed and lyophilized to yield DVP sample. The percentage polysaccharide yield (%) is calculated as follows:

$$\text{MLCP extraction yield } \%(\text{w/w}) = \frac{W_o}{W} \quad (1)$$

W_o (g) is the dried DVP weight; W (g) is the dried powder of *D. viscosa* leaf weight.

2.2.2. Experimental design of RSM

A five-level-three-factor, central composite rotatable design (CCRD) was employed in this optimization study. Extraction time (X_1), extraction temperature (X_2) and ultrasonic power (X_3) were chosen for independent variables to be optimized for the extraction of DVP. The extraction yield of polysaccharide (Y) was taken as the response of the design experiments. Twenty experiments were carried out in CCRD (Table 1). Five replicates at the center point were used for estimation of a pure error sum of squares. Triplicate determinations were performed at all design points in randomized order. A quadratic polynomial model was fitted to correlate the response variable (extraction yield of polysaccharide (%)) to the independent variables. The general form of quadratic polynomial equation is as follows:

$$Y(\%) = \beta_i + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum_{i<j=2}^{3}\beta_{ij}X_iX_j \quad (2)$$

where Y is the response variable, and β_o, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linearity, square, and interaction, respectively, while X_i and X_j are the independent variables.

The uncoded independent variables ($X_1, X_2, \ldots, X_k$) are coded in accordance with the transfer equation given below (Adinarayana & Ellaiah, 2002):

$$x_i = \frac{x_i - x_o}{\Delta X_i} \quad i = 1, 2, 3, \ldots, k \quad (3)$$

where x_i is the dimensionless value of an independent variable; X_i is the real value of an independent variable; X_o is the real value of an independent variable at the center point; and ΔX_i is the step change of the real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i.

The statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of the model was checked accounting for R^2, adjusted-R^2 and PRESS in Eqs. (4)–(6), respectively (Gharibzahedi et al., 2012):

$$R^2 = 1 - \frac{\text{SS}_{\text{Residual}}}{\text{SS}_{\text{Residual}} + \text{SS}_{\text{Model}}} \quad (4)$$

$$R^2_{\text{Adj.}} = 1 - \frac{\text{SS}_{\text{Residual}}/\text{DF}_{\text{Residual}}}{(\text{SS}_{\text{Residual}} + \text{SS}_{\text{Model}})/(\text{DF}_{\text{Residual}} + \text{DF}_{\text{Model}})} \quad (5)$$

$$\text{PREES} = \sqrt{\sum_{i=1}^{N}(y_{\text{Pred},i} - y_{\text{Exp},i})^2} \quad (6)$$

Adequate precision compares the range of the predicted values at the design points to the average prediction error. The definition of adequate precision is in Eqs. (7) and (8):

$$\text{adequate precision} = \frac{\text{Max}(\bar{y}) - \text{min}(\bar{y})}{\sqrt{\nabla(\bar{y})}} \quad (7)$$

$$\bar{V}(\bar{y}) = \frac{1}{n}\sum_{i=1}^{N}V(\bar{y}) = \frac{p\sigma^2}{n} \quad (8)$$

In Eqs. (4)–(8), SS is the sum of squares, DF is the degrees of freedom, $y_{\text{Exp},i}$ is the experimental responses, $y_{\text{Pred},i}$ the predicted responses, $\bar{y}$ is the predicted value, p is the number of model parameters, σ^2 is the residual mean square from ANOVA table, and n is the number of experiments.

Numerical and graphical optimization technique of the Design Expert software was used for simultaneous optimization of the multiple responses. The desired goal for variables and response was chosen. All the independent variables were kept within range while the response was maximized.

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)	30	40	50	60	70
Extraction temperature (°C)	55	65	75	85	95
Ultrasonic power (W)	100	200	300	400	500

2.3. Antioxidant activities

2.3.1. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of DVP was performed by the method of Xiao et al. (2012): 1.0 mL samples of different concentration (30, 60, 90, 120, 150, 180, 210 and 240 μg/mL) were mixed with 0.2 mmol/L crystal violet (1.0 mL), 1 mmol/L $FeSO_4$ (1.0 mL), 0.12% H_2O_2 (1.0 mL) and 6.0 mL sodium phosphate buffer

(pH 4.5) for 30 min, the absorbance at 578 nm was recorded as A_x, the absorbance with 1.0 mL water instead of sample was recorded as A_b, the absorbance with 1.0 mL water instead of H_2O_2 solution was recorded as A_o. Butylated hydroxytoluene (BHT) was used as a positive control. The hydroxyl radical scavenging effect was calculated as follows:

$$\text{Hydroxyl radical scavenging activity } (\%) = \frac{A_x - A_b}{A_o - A_b} \times 100 \quad (9)$$

2.3.2. 1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The DPPH radical-scavenging effect of DVP was investigated according to the method described by Lai, Wen, Li, Wu, and Li (2010). Briefly, polysaccharide samples were dissolved in methanol. One milliliter of sample solution with different concentration (30, 60, 90, 120, 150, 180, 210 and 240 μg/mL) of the samples was thoroughly mixed with 2 mL of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min in the dark, and the absorbance was then measured at 517 nm by ultraviolet–visible spectrophotometer. Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity. 95% ethanol was used as the blank control, and ascorbic acid was used as a positive control. The ability to scavenge the free radical DPPH in percentage of sample was calculated according to the following equation:

$$\text{scavenging activity } (\%) = 1 - \frac{A_{\text{sample+DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \quad (10)$$

where A_{DPPH} is the absorbance of DPPH without sample, A_{sample} is the absorbance of sample without DPPH, and $A_{\text{sample+DPPH}}$ is the absorbance of sample and DPPH.

3. Results and discussion

3.1. Effect of extraction time on extraction yield of DVP

The extraction yield (%) of DVP affected by different extraction time (20, 30, 40, 50, 60, 70, 80, 90 and 100 min) was seen in Fig. 1a, when the other two factors (extraction temperature and ultrasound power) were fixed at 75 °C and 300 W. The extraction yields of the DVP (%) significantly increased from 6.0 to 8.8% as time of extraction increased from 20 to 70 min, and then the extraction yield of DVP no longer obviously changed, when the extraction time increasing. It was reported that a long extraction time favors the production of polysaccharides (Hou & Chen, 2008; Liu, Wei, Guo, & Kennedy, 2006). This might be due to the time requirement of the exposure of the polysaccharides to the release medium where the liquid penetrated into the dried raw material, dissolved the polysaccharides and subsequently diffused out from the raw material. In addition, increasing extraction temperature would increase the solubility of the extracted polysaccharides, giving a higher rate of extraction. Therefore, extraction time range of 30–70 min was adopted in the present work.

3.2. Effect of extraction temperature on extraction yield of DVP

Different extraction temperature was set at 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 °C, respectively, to investigate the influence of extraction temperature on the extraction yield of DVP (%) when the other factors were set as follows: extraction time 50 min and ultrasound power 300 W. Fig. 1b indicated that the extraction yield of DVP increases with the increasing extraction temperature and reached the peak value from 95 to 100 °C. And

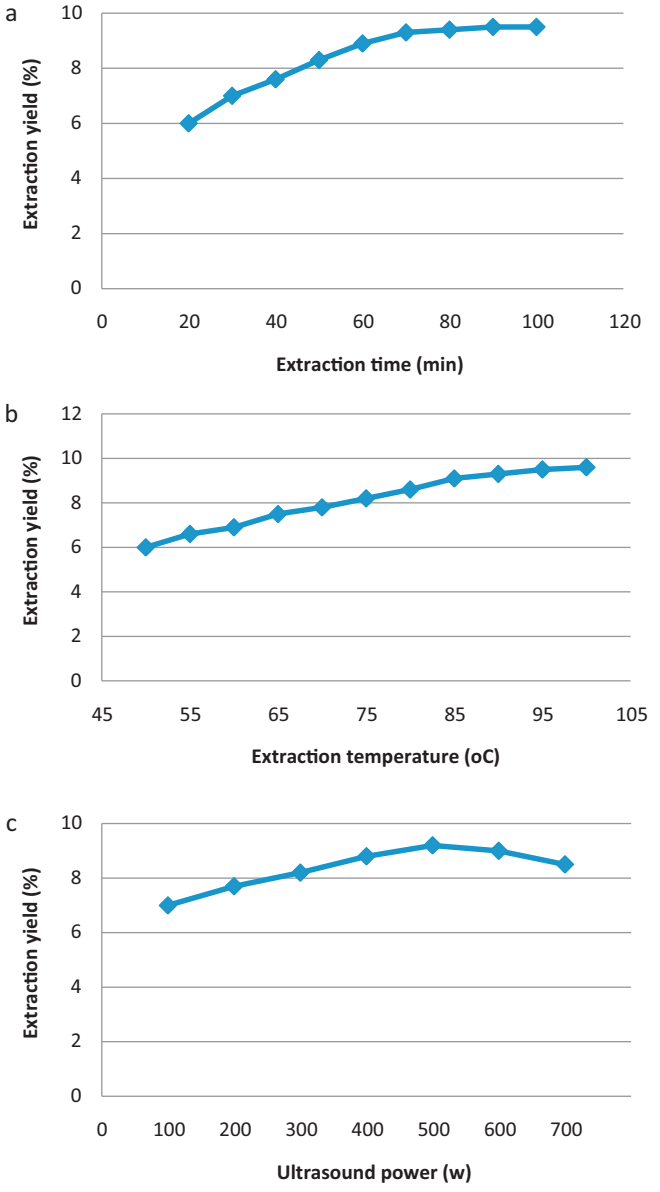

Fig. 1. Effects of different (a) temperatures, (b) times and (c) ultrasound power on extraction yield of DVP (%).

then there is no increase when extraction temperature continued to rise. The extraction coefficient increased with increasing the extraction temperature due to the increase of the polysaccharides solubility (Braga, Moreschi, & Meireles, 2006). It has also been cited that the extraction at the elevated temperature resulted in faster and easier mass transfer of water-soluble polysaccharide from the cell wall into the extract (Tabatabaee Amid & Mirhosseini, 2012). 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 industrialization point of view (Ye & Jiang, 2011). Therefore, 55–95 °C was considered in this experiment.

3.3. Effect of ultrasound power on extraction yield of DVP

The effect of ultrasound power (100, 200, 300, 400, 500, 600 and 700 W) on the extraction yield of DVP (%) were studied when the other two factors (extraction time and extraction temperature)

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

Run	Coded variables			Actual variables			Extraction yield (%)		
	X_1 (min)	X_2 (°C)	X_3 (W)	X_1 (min)	X_2 (°C)	X_3 (W)	Experimental	Predicted	Residue
1	0	0	0	50	75	300	6.00	5.79	0.21
2	0	0	0	50	75	300	7.80	7.84	−0.04
3	0	0	(+2)	50	75	500	7.40	7.49	−0.09
4	(−1)	(+1)	(−1)	40	85	200	8.50	8.54	−0.04
5	(+1)	(+1)	(+1)	60	85	400	8.10	8.14	−0.04
6	0	(+2)	0	50	95	300	9.20	9.19	0.01
7	(−1)	(+1)	(+1)	40	85	400	9.10	9.14	−0.04
8	(−1)	(−1)	(+1)	40	65	400	8.90	9.19	−0.29
9	(+1)	(−1)	(−1)	60	65	200	6.20	6.26	−0.06
10	(−1)	(−1)	(−1)	40	65	200	8.50	8.36	0.14
11	0	0	(−2)	50	75	100	7.50	7.61	−0.11
12	0	0	0	50	75	300	9.50	9.31	0.19
13	0	0	0	50	75	300	7.10	7.16	−0.06
14	(+1)	(+1)	(−1)	60	85	200	10.30	10.16	0.14
15	0	0	0	50	75	300	8.30	8.24	0.06
16	(+2)	0	0	70	75	300	8.10	8.24	−0.14
17	0	(−2)	0	50	55	300	8.20	8.24	−0.04
18	(+1)	(−1)	(+1)	60	65	400	8.40	8.24	0.16
19	0	0	0	50	75	300	8.20	8.24	−0.04
20	(−2)	0	0	30	75	300	8.30	8.24	0.06

were fixed at 50 min and 75 °C. Fig. 1c shows ultrasonic sound had a significant effect on the extraction yield of DVP (%). When the power was increased from 100 to 500 W, the extraction yield of DVP (%) increased significantly and almost linearly from 7.1 to 9.3%, and then decreased when the extraction power was over 500 W. Sonication is widely used for extraction of various substances from plant material and generates microscopic bubbles (Tian, Xu, & Zheng, 2013; Vilkhu et al., 2008; Vinatoru, 2001). The type and number of bubbles created and collapsed are positively correlated to the amplitude of ultrasonic waves traveling through the solvent; the collapsing bubbles are believed to create high-shear gradients by causing microstreaming which disrupts the cell walls (Gogate & Kabadi, 2009; Jaki, Franzblau, Cho, & Pauli, 2006). This significantly accelerates the penetration of solvent into cells and the release of components from cells into the solvent, and simultaneously significantly enhances the mass transfer rate (Lou, Wang, Zhang, & Wang, 2010; Tian et al., 2013). It is also reported that acoustic cavitation in ultrasonic-assisted extraction yielded hydroxyl radicals, leading to chemical decomposition (Koda, Kimura, Kondo, & Mitome, 2003; Li, Pordesimo, & Weiss, 2004). More chemical decompositions were generated with the stronger ultrasound power. In this study the extraction yield of polysaccharides decreased after 500 W. Thus, 500 W was chosen as the optimum extraction power.

According to the single-parameter study, we adopted extraction time 30–70 min, extraction temperature 55–95 °C, and ultrasound power 100–500 W for RSM experiments.

3.4. Statistical analysis and modeling of extraction of polysaccharides

The fitted model for extraction yield of DVP (%) to predict the relationship between the independent variables and the dependent variable can be expressed by:

$$\begin{aligned}\text{Extraction yield}\,(\%) = {} & -18.6008 + 0.54681 * X_1 + 0.1364X_2 \\ & + 0.0267X_3 - 0.0025X_1X_2 - 0.0002X_1X_3 \\ & - 0.0001X_2X_3 - 0.0023X_1^2 + 0.0005X_2^2 \\ & + 0.00001X_3^2 \end{aligned} \quad (11)$$

The process variables and experimental data for extraction yield of DVP under different treatment conditions are presented in Table 2.

Analysis of variance (ANOVA) was performed to investigate the adequacy of the suggested model (Eq. (11)) and identify the significant factors. The results of the analysis of variance, adequacy and fitness of the models are summarized in Table 3. A significant lack of fit shows that the models failed to represent the data in the experimental domain at which points were not included in the regression. The ANOVA showed that lack of fit was not significant for response surface model at 95% confidence level, which means that the model represented the data satisfactorily. On the other hand, R^2, adj-R^2, PRESS, coefficient of variation (CV) and adequate precision were

Table 3
Analysis of variance for the fitted models.

Source	DF	Coefficient	Sum of square	Mean square	*F*-value	*P*-value
Extraction yield (%)						
Model	9		19.697	2.189	74.305	<0.0001
Residual	10		0.295	0.029		
Lack of fit	5		0.240	0.048	4.355	0.0661 ns
Pure error	5		0.055	0.011		
Total	19		19.992			
R^2		0.985				
Adj-R^2		0.972				
CV		2.098				
PRESS		2.008				
Standard deviation		0.172				
Adequate precision		35.995				

Table 4
The significance of each response variable effect showed by using *F* ratio and *P* value in the nonlinear second order model.

Variables	DF[a]	SS[b]	MS[c]	*F*-value	*P*-value[d]
Linear effects					
X_1	1	4.410	4.410	149.722	<0.0001
X_2	1	2.890	2.890	98.117	<0.0001
X_3	1	9.000	9.000	305.556	<0.0001
Quadratic effects					
X_1^2	1	1.351	1.351	45.87302	<0.0001
X_2^2	1	0.078	0.078	2.646605	0.1348 ns[e]
X_3^2	1	0.281	0.281	9.53373	0.0115
Interaction effects					
X_1X_2	1	0.500	0.500	16.97531	0.0021
X_1X_3	1	0.500	0.500	16.97531	0.0021
X_2X_3	1	0.245	0.245	8.317901	0.0163

[a] Degrees of freedom.
[b] Sum of squares.
[c] Mean sum of squares.
[d] *P*-values <0.05 were considered to be significant.
[e] ns: not significant.

calculated to check the model adequacy. The data indicated that the proposed regression model for extraction yield was adequate with satisfactory R^2 (determination coefficient) and adj-R^2 values. A high R^2 indicates that the variation could be accounted for by the data satisfactorily fitting the model. However, a large value of R^2 does not always imply that the regression model is a good one. Adding a variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, it is better to use an adj-R^2 to evaluate the model adequacy. The R^2 and adj-R^2 values for extraction yield of DVP were 0.985 and 0.972, respectively, which showed a close agreement between the experimental results and the theoretical values predicted by the polynomial model. The CV values were found to be 2.098 for yield extraction of DVP. Since CV is a measure expressing the standard deviation as a percentage of the mean, smaller values of CV give better reproducibility. In general, a CV higher than 10 indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model (Myers & Montgomery, 2002). The low PRESS 2.008 value suggests for the adequacy of the fitted quadratic models for predictive applications. Adequate precision measures the signal-to noise ratio. A ratio greater than 4 is desirable (Myers & Montgomery, 2002). For the proposed models, this value was 35.995, a very good signal-to-noise ratio. All these statistical parameters show the reliability of the models. The *P*-values are used as a tool to check the significance of each coefficient (Table 4). The smaller the value of *P*, the more significant is the corresponding coefficient. From the model of extraction yield (Eq. (11)), linear terms of extraction time (X_1), extraction temperature (X_2) and ultrasonic power (X_3), quadratic terms of extraction time (X_1^2) and extraction temperature (X_2^2) were significant ($P < 0.05$). All of the interaction terms, X_1X_2, X_1X_3 and X_2X_3 were significant ($P < 0.05$).

3.5. Perturbation plot

Perturbation plot shows the comparison between all factors at a selected point in the considered design space. The perturbation plot for the extraction yield of DVP is shown in Fig. 2. The extraction yield response was drawn by changing only one factor over its range while the other factors were held constant. The plot demonstrates the effect of all factors at a central point in the design space (e.g., extraction time, extraction temperature and ultrasound power). All factors indicated a positive effect on the extraction yield of DVP. The relatively flat line of temperature shows lower effect of this factor on the extraction yield of DVP in the design space. It can be seen from Eq. (11) and Table 4 the perturbation plot that

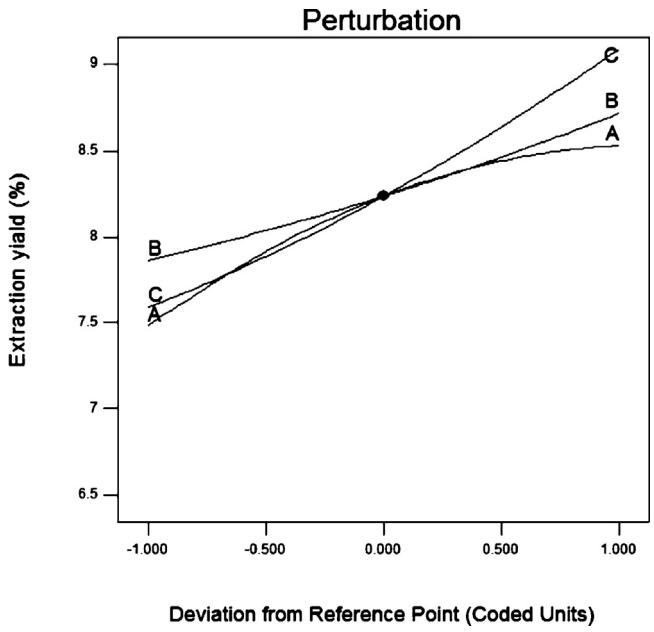

Fig. 2. Perturbation plot for rate response (for A: extraction time, B: extraction temperature and C: ultrasound power).

extraction time and ultrasound power had significant curvature effect. The steep curvature in extraction time behavior demonstrated the response of extraction yield of DVP% was very rapid to these factors. Through the comparison of coefficients in Eq. (11), the most significant parameter was determined. In this manner, the order of positive influence of the individual terms on the obtained extraction yield response was ultrasound power, extraction time and extraction temperature.

3.6. Optimization of extraction conditions of DVP

In the present study, response surfaces were plotted by Design-Expert software to research the effects of parameters and their interactions on the yield of polysaccharides. Figs. 3–5 show three independent response surface plots and their respective contour plots, which are useful to know about interaction effects of these factors on the responses. In the 3-D response surface plot and contour plot, the purity of DVP was obtained along with two variables, while the other variable was fixed constant at its respective 0 level (center value of the testing ranges). In Figs. 3–5, 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, Chirumamil, Marchant, & Nigam, 2001). The independent variables and maximum predicted values from the figures corresponded with the optimum values of the dependent variables obtained by the equations.

Fig. 3a and b shows the effects of extraction temperature (X_1) and extraction time (X_2) on the yield of DVP. With the ultrasonic power set at 300 W, the highest DVP should be found with an extraction temperature range of 65–85 °C and an extraction time range of 40–60 min.

Fig. 4a and b shows the effects of extraction temperature (X_1) and ultrasonic power (X_3) on extraction yield of DVP are shown in. The yield of DVP increases with an increase in extraction temperature and ultrasonic power. With the treatment time set at 50 min, the highest DVP yield should be found with an extraction temperature and ultrasonic power range of 65–85 °C and 200–400 W, respectively.

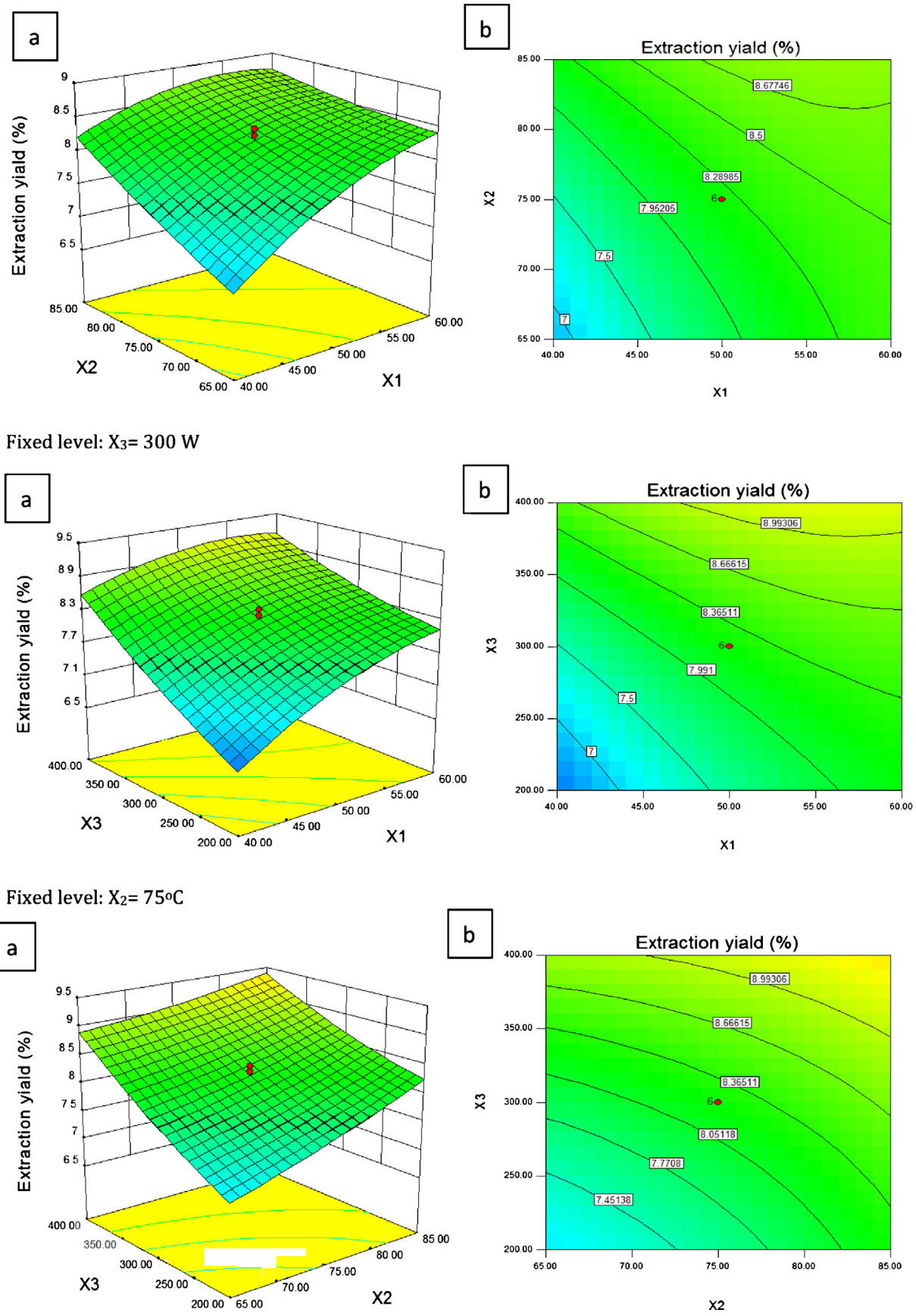

Fig. 3. Response surface (3-D) and contour plots showing the effects of the extraction temperature (X_1), extraction time (X_2) and ultrasonic power (X_3) on the DVP extraction yield.

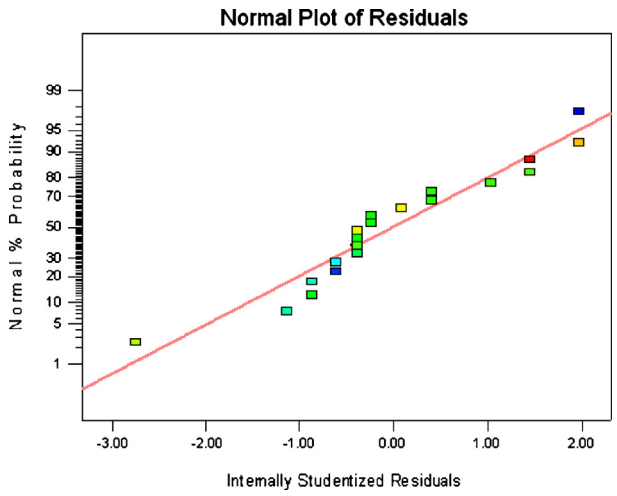

Fig. 4. Normal probability of internally studentized residuals.

Fig. 5a and b shows the effects of extraction time (X_2) and ultrasonic power (X_3) on the extraction yield of DVP. With the extraction temperature set at 75 °C, the optimal DVP yield should be found with an ultrasonic power range of 200–400 W with extraction time around 40–60 min.

It can be concluded that optimal extraction conditions of DVP was extraction time 50.54 min, extraction temperature 85 °C and ultrasound power 400 W.

3.7. Model adequacy checking

Usually, it is necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with the investigation and optimization of the fitted response surface likely give poor or misleading results (Li et al., 2004). The residuals from the least squares fit play an important role in judging model adequacy (Myers & Montgomery, 2002). By constructing a normal probability plot of the residuals, a check was made for the normality assumption, as given in Fig. 4. The normality assumption was satisfied as the residual plot approximated along a straight line. Fig. 5 presents a plot of residuals versus the predicted response. The general impression is that the residuals scatter randomly on the display, suggesting that he variance of the original observation is constant for all values of *Y*. Both of the plots (Figs. 4 and 5) are satisfactory, so we conclude that the empirical model is adequate to describe the DVP extraction yield by response surface.

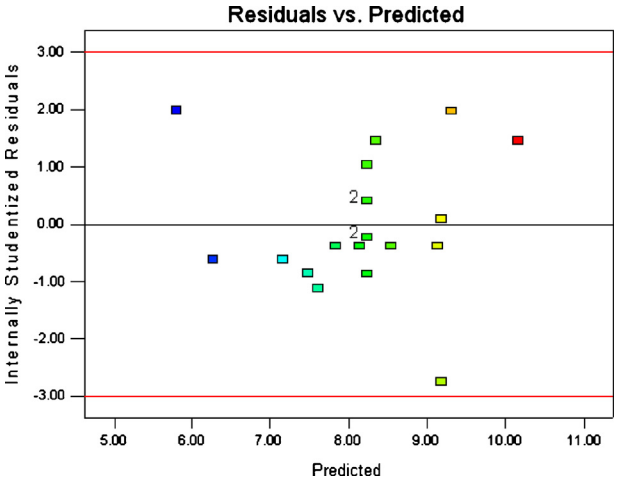

Fig. 5. Plot of internally studentized residuals versus predicted response.

3.8. Verification of predictive model

The suitability of the model equation for predicting optimum response value was tested under the conditions: extraction time 50.54 min, extraction temperature 85 °C and ultrasound power 400 W. This set of conditions was determined to be optimum by the RSM optimization approach and was also used to validate experimentally and predict the value of the response using the model equation. A mean value of 9.455 ± 0.24 (n = 4), obtained from real experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process (Table 5).

3.9. Antioxidant activity of DVP

3.9.1. DPPH scavenging activity of DVP

The change of concentration of DVP was monitored to evaluate the antioxidant ability of DVP through the DPPH scavenging activity test (Fig. 6a). As shown in Fig. 6a, DVP exhibited a concentration dependent antiradical activity by inhibiting DPPH free radicals. The DPPH scavenging effect increased by increasing the concentration of DVP up to 240 μg/mL. The results indicated that DVP had a noticeable effect on scavenging DPPH free radicals, especially at high concentrations. However, the radical-scavenging activity of DVP (64%) was lower than that of BHT (73%) used in this study. The possible mechanism by which DVP acts as an antioxidant may be attributed to their electron donation power to the free radicals, thereby terminating the radical chain reaction (Lai et al., 2010).

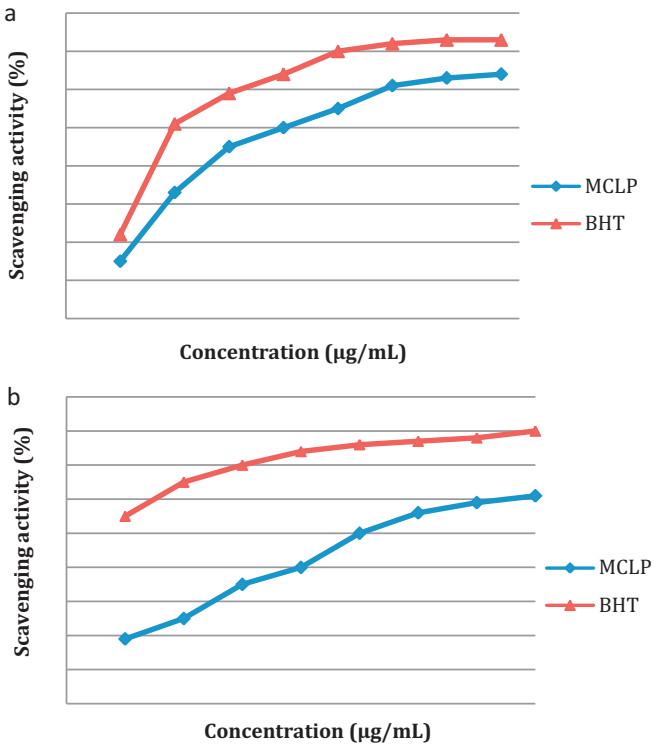

Fig. 6. (a) Scavenging effect of DVP on DPPH radicals compared with that of butylated hydroxytoluene (BHT) (b) Scavenging activity of DVP on hydroxyl radicals compared with that of ascorbic acid and.

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

Optimum condition			Extraction yield of DVP (%)	
Extraction time (min)	Extraction temperature (°C)	Ultrasonic power (W)	Experimental	Predicted
50.54	85	400	9.455 ± 0.24[a]	9.398

[a] Mean ± standard deviation ($N = 4$).

3.9.2. Scavenging activity of hydroxyl radicals

Among the reactive oxygen species, Hydroxyl radical is the most active free radical that attacks all the biological molecules by setting off free radical chain reactions (Barry & Susanna, 1993). Hydroxyl radicals can react with almost all the biomacromolecules functioning in living cells and induce severe damage to the adjacent biomolecules (Rollet-Labelle et al., 1998). Therefore, the removal of hydroxyl radical is important for antioxidant defense in cell or food systems. DVP was found to have the ability to scavenge hydroxyl radicals at concentration 50–240 μg/mL (Fig. 6b). Interestingly, the scavenging ability of DVP on hydroxyl radicals was in a concentration-dependent manner. The inhibition percentage of DVP at 240 μg/mL reached 61%, which was 23.75% lower than that of BHT (80%).

4. Conclusion

An efficient ultrasonic extraction technique was employed to extract polysaccharide from the leaves of *D. viscosa* (DVP) by RSM. Central composite rotatable design (CCRD) was used to determine the optimum process parameters that could give a high extraction yield. A second-order polynomial model was employed to optimize the extraction of DVP by ultrasonic technology. The optimal conditions determined are as follows: extraction temperature, 50 °C; extraction time, 30 min; and ultrasonic power 400 W. Under these optimal conditions, a maximum polysaccharide extraction yield of 9.398% can be achieved. A mean value of 9.455 ± 0.24, obtained from real experiments, demonstrated the validation of the RSM model, indicating that the model was adequate for the extraction process. In addition, the anti-oxidative activity of DVP was investigated by measuring its scavenging ability on DPPH and hydroxyl radicals. The results indicated that DVP has good antioxidant activity.

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