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Optimization of extraction process of crude polysaccharides from boat-fruited sterculia seeds by response surface methodology

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Abstract

Response surface methodology (RSM) was applied to optimize the extraction of crude polysaccharides from boat-fruited sterculia seeds. A central composite design was used for experimental design and analysis of the results to obtain the optimal extraction conditions. Extraction temperature, pH, extraction time and water to seed ratio were found to have a significant influence on the yield and purity of the extracted crude polysaccharides, while the three other factors except the water to seed ratio also significantly affected the relative viscosity. Based on the RSM analysis, optimum conditions were: temperature 60–65 °C, time 2.3–3.1 h, pH at 7.0 and water to seed ratio at 75:1. Under the optimized conditions, the experimental values were in close agreement with values predicted by the model. The crude polysaccharides prepared under optimum conditions contained 58.2% total carbohydrates (including uronic acids), 20% proteins, 9% moisture and 4.5% ash. The crude polysaccharides consisted of glucose (22.6%), rhamnose (10.0%), arabinose (7.9%), galactose (5.0%), xylose (0.8%) and galacturonic acid (11.8%).

Keywords: Boat-fruited sterculia seeds; Crude polysaccharides; Extraction; Optimization; Response surface methodology

1. Introduction

Boat-fruited sterculia seed (*Semen Sterculiae Lychnophorae*) is a tropical herb of the Sterculiaceae family, mainly distributed in Vietnam, Thailand, Malaysia, Indonesia as well as South China (Wang et al., 2003). This plant is commonly used for the treatment of many diseases, such as resolving phlegm (by "clearing heat from the lungs" as explained in Chinese medicine) and relieving sore throat to restore the voice on the upper respiratory tract, relaxing the bowels to relieve constipation, etc. (Xiao, 2002). It has been used for hundreds of years in traditional Chinese medicinal prescriptions because of its pharmacological effects.

In the past several years, medicinal plant polysaccharides have been widely studied for their chemical properties and biological activities (Sun, Tang, Gu, & Li, 2005; Wu, Zhao, & Qin, 2002). However, little information is available about the polysaccharides from boat-fruited sterculia seeds (Chen, Cao, & Song, 1996; Chen, Li, Shen, Peng, & Xu, 1994). The content of polysaccharides in the seeds varies from 7.61% to 12.55% depending on genetic and environmental factors (Chen et al., 1994). The seeds harvested in Southeast Asia usually contain more polysaccharides than those in China, possibly due to the tropical climate. Aqueous extractions are the most common methods used for the extraction of the plant polysaccharides. Preliminary tests in our laboratory showed that extraction temperature, pH, extraction time and water to seed ratio might have significant influence on the yield, purity and relative viscosity of the crude polysaccharides. Thus, it is

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important to optimize the extraction process in order to obtain high yield and high quality polysaccharides (purity and relative viscosity).

The classical method of studying one variable at a time may be effective in some processes, but fails to consider the combined effects of several factors involved. In extraction processes, where there are multiple independent variables affecting the responding factors, it is likely that the operational variables interact and influence each other's effects on the response. Therefore, it is necessary to use an optimization method that can determine all the factors as well as the possible interactions between these independent variables, so that a set of optimal experimental conditions can be determined (Cui, Mazza, Oomah, & Biliaderis, 1994). Optimization through factorial design and response surface analysis particularly fulfills this requirement. Response surface methodology (RSM) is a collection of mathematical and statistical techniques widely applied in the food industry to determine the effects of several variables and optimize conditions (Ginevra, Beatriz, Altero, & Francisco, 2002; Levigne, Ralet, & Thibault, 2002; Li & Fu, 2005; Tanyildizi, Özer, & Elibol, 2005; Vohra & Satyanarayana, 2002). The main advantage of RSM is to reduce the number of experimental trials needed to evaluate multiple variables and its interactions, it is less laborious and time-consuming than other approaches (Wu, 2002). In general, it applies an experimental design such as central composite design to fit a second order polynomial by a least squares technique. An equation is used to describe how the test variables affect the response and determine the interrelationship among the variables. The objective of the present work was to optimize and study the effect of extraction temperature, pH, extraction time and water to seed ratio on the aqueous extraction yield, purity and relative viscosity of crude polysaccharides from boat-fruited sterculia seeds using RSM.

2. Materials and methods

2.1. Samples and chemicals

The boat-fruited sterculia seeds harvested in Vietnam were provided by Shanhe Pharmaceutical Co. Ltd. (Wuxi, China). All chemicals used were reagent grade unless otherwise specified.

2.2. Extraction of crude polysaccharides

All samples (seeds) tested were ground in a high speed disintegrator (Model SF-2000, Chinese Traditional Medicine Machine Works, Shanghai, China) to obtain a fine powder (Particle size: 420 μm), then were defatted in a Soxhlet apparatus with petroleum ether (30–60 °C), and pretreated with 80% ethanol 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 (Chen et al., 1996).

The pretreated dry powder (20.0 g) was extracted with deionized water (water–seed ratio (ml/g) ranging from 45:1 to 105:1) at pH 3.5–9.5 (adjusting the suspension pH by 0.1 mol/l NaOH or HCl), while the temperature of the water bath ranged from 30 to 90 °C and was kept steady (within ±1.0 °C). The water-seed slurry in a 3.0 l stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 0.5 to 4.5 h) during the entire extraction process. The mixture was centrifuged (2000*g*, 20 min), then the supernatant was separated from insoluble residue with nylon cloth (Pore diameter: 38 μm). The extracts were precipitated by the addition of ethanol to a final concentration of 75% (v/v), and the precipitates were collected by centrifugation (2000*g*, 20 min), then solubilized in deionized water and lyophilized to get the crude polysaccharides.

2.3. Analytical methods

The yield of crude polysaccharides was calculated as a percentage of the weight of the seeds pretreated dry powder. The sugar content was determined by the reaction of sugars with phenol in the presence of sulfuric acid using glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The total uronic acid content was colorimetrically determined by the *m*-hydroxydiphenyl assay (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid as a standard. The relative viscosity (relative to deionized water) of the crude polysaccharides was determined on an Ubbelohde Viscometer (Yatai Glass, Shanghai, China) at 25.0 °C. Moisture and ash were determined according to AOCS and AOAC methods (AOCS, Ba 2a-38; AOAC 942.05), while the protein content was determined using the Kjeldahl method with a conversion factor of 6.25 (AOCS Ba 4a-38). The monosaccharide composition was performed by gas chromatography (GC-14A, Shimadzu, Japan) (Erbing, Jansson, Widmalm, & Nimmich, 1995). The polysaccharides were hydrolyzed by 2 M trifluoroacetic acid (at 121 °C, for 1 h) into monosaccharides, and were detected with a flame ionization detector (FID). The type of uronic acid was distinguished by enzyme hydrolysis of Driselase (Sigma–Aldrich) on a Dionex HPAEC system equipped with pulsed amperometric detection using galacturonic acid as standard (Sunnyvale, CA) (Nakamura, Furuta, Maeda, Takao, & Nagamatsu, 2002).

2.4. Experimental design

To explore the effect of independent variables on the response within the range of investigation, a central composite design (CCD) with four independent variables (X_1, extraction temperature; X_2, suspension pH; X_3, extraction time; X_4, water to solid ratio) at five levels was performed (Haaland, 1989). For statistical calculation, the variables were coded according to

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad (1)$$

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 are 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. Seven replicates (treatment 25–31) at the centre of the design were used to allow for estimation of a pure error sum of squares.

2.5. Statistical analysis

The quadratic model for predicting the optimal point was expressed according to

$$Y_k = \beta_{k_0} + \sum_{i=1}^{4} \beta_{k_i} x_i + \sum_{i=1}^{4} \beta_{k_{ii}} x_i^2 + \sum_{i<j=2}^{4} \beta_{k_{ij}} x_i x_j \quad (2)$$

where Y_k is the response function, β_{k_0} is the centre point of the system, β_{k_i}, $\beta_{k_{ii}}$ and $\beta_{k_{ij}}$ represent the coefficients of the linear, quadratic and interactive terms, respectively; x_i, x_{ii} and $x_i x_j$ represent the linear, quadratic and interactive terms of 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 (Triveni, Shamala, & Rastogi, 2001). The analysis of variance tables were generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of all terms in the polynomial were judged statistically by computing the F-value at a probability (p) of 0.001, 0.01 or 0.05. The regression coefficients were then used to make statistical calculation to generate dimensional and contour maps from the regression models. The response surface regression (RSREG) procedure of statistical software package (SAS System V8) was used to analyze the experimental data.

Table 1
The range of independent variables and their corresponding levels

Independent variables	Symbol		Coded factor level				
	Uncoded	Coded	−2	−1	0	1	2
Extraction temperature (°C)	X_1	x_1	30	45	60	75	90
pH	X_2	x_2	3.5	5.0	6.5	8.0	9.5
Extraction time (h)	X_3	x_3	0.5	1.5	2.5	3.5	4.5
Water to seed ratio (ml/g)	X_4	x_4	45:1	60:1	75:1	90:1	105:1

3. Results and discussion

3.1. Statistical analysis and the model fitting

The experimental data for yield, purity and relative viscosity of the crude polysaccharides under different treatment conditions are presented in Table 2. After the RSREG procedure, the effect of treatment variables as the linear, quadratic and interaction terms were tested for adequacy and fitness by analysis of variance (ANOVA) (Table 3). The statistical analysis indicated that the proposed regression model for yield, purity and relative viscosity was adequate, possessing no significant lack of fit and with satisfactory values of the R^2 (multiple correlation coefficient) for all the responses. The R^2 values were 0.987, 0.978 and 0.961 for yield, purity and relative viscosity, respectively (Table 3). The closer the value of R^2 to the unity, the better the empirical model fits the actual data (Lee, Yusof, Hamid, & Baharin, 2006).

The regression coefficients of all the three models were obtained and tested for adequacy and fitness by analysis of variance (Table 4). From the model of yield, linear

Table 2
The central composite experimental design and results for extraction yield, purity and relative viscosity of crude polysaccharides

Run order	Temperature x_1	pH x_2	Time x_3	Water: seed x_4	Yield[a] (%) Y_1	Purity (%) Y_2	Relative viscosity Y_3
1	−1	−1	−1	−1	11.81	53.12	2.01
2	−1	−1	−1	1	12.84	55.43	2.18
3	−1	−1	1	−1	13.03	52.66	2.05
4	−1	−1	1	1	14.64	55.03	2.24
5	−1	1	−1	−1	12.06	54.32	1.83
6	−1	1	−1	1	13.51	55.75	1.98
7	−1	1	1	−1	13.66	52.96	1.96
8	−1	1	1	1	15.23	54.74	2.14
9	1	−1	−1	−1	15.26	54.63	1.46
10	1	−1	−1	1	15.71	53.22	1.53
11	1	−1	1	−1	16.88	53.91	1.52
12	1	−1	1	1	17.34	52.63	1.66
13	1	1	−1	−1	16.33	55.61	1.30
14	1	1	−1	1	17.45	53.42	1.34
15	1	1	1	−1	18.14	54.54	1.39
16	1	1	1	1	19.02	52.42	1.41
17	−2	0	0	0	10.24	48.83	2.04
18	2	0	0	0	18.06	49.11	1.12
19	0	−2	0	0	15.15	58.22	1.68
20	0	2	0	0	17.52	57.12	1.70
21	0	0	−2	0	13.14	53.74	1.80
22	0	0	2	0	17.92	52.83	2.13
23	0	0	0	−2	13.63	54.55	2.04
24	0	0	0	2	17.02	55.02	2.12
25	0	0	0	0	17.21	56.13	2.16
26	0	0	0	0	17.63	56.42	2.18
27	0	0	0	0	17.42	56.21	2.21
28	0	0	0	0	17.07	56.11	2.15
29	0	0	0	0	17.55	56.22	2.26
30	0	0	0	0	17.54	56.15	2.27
31	0	0	0	0	17.12	56.84	2.13

[a] Mean of duplicate runs.

Table 3
Analysis of variance for extraction variables as linear, quadratic terms and interactions on response variables

Source	D.F.[a]	Yield Y_1		Purity Y_2		Relative viscosity Y_3	
		Sum of squares	Pr > F	Sum of squares	Pr > F	Sum of squares	Pr > F
Model	14	152.87***	<.0001	128.21***	<.0001	3.32***	<.0001
Linear	4	121.95***	<.0001	3.53	0.01	2.03***	<.0001
Quadratic	4	29.47***	<.0001	109.88***	<.0001	1.27***	<.0001
Cross product	6	1.45	0.13	14.80***	<.0001	0.02	0.91
Residual	16	1.99		2.96		0.14	
Lack of fit	10	1.68	0.08	2.55	0.06	0.12	0.06
Pure error	6	0.31		0.41		0.02	
Variability explained R^2		0.987		0.978		0.961	

[a] D.F., degree of freedom.
*** Significant at 0.001 level.

Table 4
Regression coefficients of the second-order polynomial equations

Regression coefficient	Response variables		
	Yield Y_1	Purity Y_2	Relative viscosity Y_3
β_0	17.363***	56.297***	2.194***
Linear			
β_1	1.875***	−0.128	−0.276***
β_2	0.526***	0.039	−0.053*
β_3	0.939***	−0.351***	0.058**
β_4	0.640***	0.076	0.047*
Quadratic			
β_{11}	−0.829***	−1.774***	−0.167***
β_{22}	−0.282***	0.401***	−0.139***
β_{33}	−0.484***	−0.695***	−0.070***
β_{44}	−0.535***	−0.320**	−0.042*
Interaction			
β_{12}	0.226*	0.004	−0.010
β_{13}	0.018	−0.009	−0.003
β_{14}	−0.172	−0.931***	−0.026
β_{23}	0.027	−0.142	0.010
β_{24}	0.092	−0.193	−0.011
β_{34}	0.029	0.038	0.006

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

and quadratic effects of the four factors were significant ($P < 0.001$). Among the interaction terms, extraction temperature with pH was significant ($P < 0.05$) to the yield. For the purity of crude polysaccharides, the coefficients of quadratic effects, the linear effect of extraction time and the interaction effect of temperature with water to solid ratio were highly significant ($P < 0.001$ and $P < 0.01$, respectively). For the relative viscosity, the coefficients of quadratic effects except the water to solid ratio and the linear effect of extraction temperature were highly significant ($P < 0.001$). In addition, the significant contributions to the relative viscosity were also identified from the linear effects of pH, extraction time and the quadratic and the linear effects of water to solid ratio. From the three models tested, all the interaction terms except the effect of extraction temperature with water to solid ratio in the purity model and temperature with pH in the yield one exhibited no obvious significant effect.

The effects of all the independent variables on the yield and purity of crude polysaccharides were highly significant ($P < 0.001$ and $P < 0.01$) (Table 5). Based on the sum of squares, the importance of the independent variables on yield could be ranked in the following order: extraction temperature (X_1) > extraction time (X_2) > water to solid ratio (X_4) > pH (X_3); while the importance of the independent variables on purity could be ranked in the order of extraction temperature (X_1) > water to seed ratio (X_4) > extraction time (X_2) > pH (X_3). Only extraction temperature (X_1), pH (X_2) and extraction time (X_3) had significant influence on the relative viscosity. The importance order could be extraction temperature (X_1) > extraction time (X_2) > pH (X_3) > water to solid ratio (X_4).

From the above analysis, it was found that the extraction temperature and time were the most important factors

Table 5
Analysis of variance showing significance of the variables on responses

Independent variables	D.F.[a]	Yield Y_1		Purity Y_2		Relative viscosity Y_3	
		Sum of squares	Pr > F	Sum of squares	Pr > F	Sum of squares	Pr > F
Extraction temperature (°C)	5	105.27***	<.0001	104.24***	<.0001	2.63***	<.0001
pH	5	9.89***	<.0001	5.55**	0.003	0.63***	<.0001
Extraction time (h)	5	27.87***	<.0001	17.13***	<.0001	0.23**	0.0046
Water to seed ratio	5	18.62***	<.0001	17.55***	<.0001	0.12	0.0581

[a] D.F., degree of freedom.
** Significant at 0.01 level.
*** Significant at 0.001 level.

affecting the response variables (extraction yield, purity and relative viscosity of crude polysaccharides). This is because the water temperature and extraction time had strong effects on the mass transfer rate of the water-soluble polysaccharides in the cell wall (Shi, Chang, Schwarz, Wiesenborn, & Shih, 1996). In addition, the results demonstrated that the effects of pH and water to solid ratio on the overall extraction process were minor.

3.2. Optimization of the procedure

Three-dimensional response surfaces and contour plots for the responses are the graphical representations of the regression equations. The main goal of response surface methodology was to identify the optimum values of the independent variables efficiently so that the responses are maximized. In the plots two continuous variables were developed for extraction yield, purity and relative viscosity responses, while the other two variables were held constant at their respective zero level (centre value of the testing ranges). The results of yield, purity and relative viscosity affected by extraction temperature, pH, extraction time and water to solid ratio are shown in Figs. 1a–b, 2a–b and 3a–b, respectively. In the three 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, Chirumamil, Marchant, & Nigam, 2001). The independent variables and maximum predicted values from the figures correspond with the optimum values of the dependent variables (responses) obtained by the equations. According to the equations, independent variables under the optimum conditions, i.e. the optimum values for the three responses, are shown in Table 6.

Optimization of the extraction procedure was based upon the following: higher extraction yield, purity and relative viscosity of crude polysaccharides, and shorter extraction time and smaller water to solid ratio which favor the reduction of energy consumption and process waste (Shi et al., 1996). In addition to the importance order of the independent variables listed in Table 5 and the above mentioned extraction efficiency, it should also be kept in mind that high temperature and low pH could result in partial hydrolysis of polysaccharides; in contrast, high pH over extended time could lead to β-elimination of the pectic material. As a result the pH and water to solid ratio were held at 7.0 and 75:1, respectively for optimizing the other two parameters. The overlaying plot attained is shown in Fig. 4. For the extraction temperature of 60–65 °C and extraction time of 2.3–3.1 h, a small shaded area, namely overlay area of the three responses is assigned as the optimum area of extraction which represents a higher amount of yield (>17.6%), high purity (>55.6%) and relative viscosity (>2.1) (pH at 7.0 ($X_2 = 0.33$) and water to solid ratio at 75:1 ($X_4 = 0$)). These results demonstrated that crude polysaccharides with higher yield, purity and relative viscosity could be obtained under the optimum extraction conditions: i.e. at temperature 60–65 °C for 2.3–3.1 h (pH at 7.0 and water to solid ratio at 75:1).

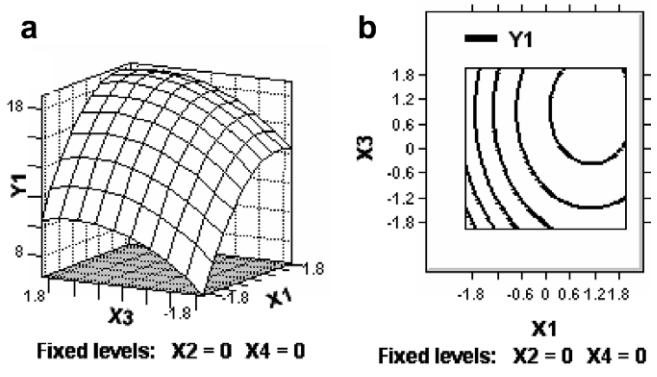

Fig. 1. Response surface (a) and contour (b) plots showing the effect of extraction temperature and extraction time (pH 6.5 and water to seed ratio 75:1) on extraction yield (Y1) of crude polysaccharides.

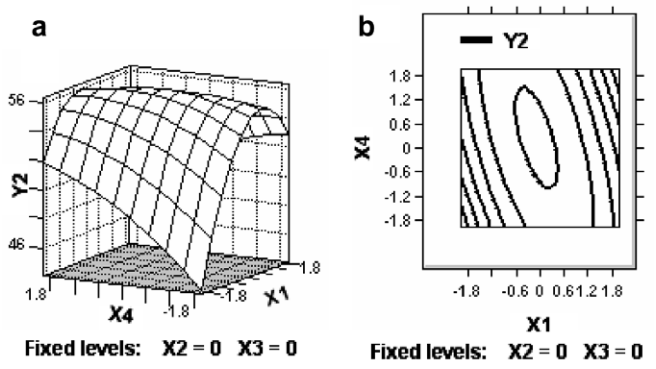

Fig. 2. Response surface (a) and contour (b) plots showing the effect of extraction temperature and water to seed ratio (pH 6.5 and extraction time 2.5 h) on purity (Y2) of the extracted crude polysaccharides.

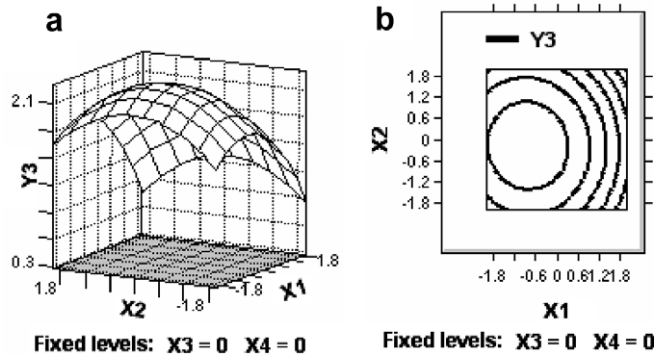

Fig. 3. Response surface (a) and contour (b) plots showing the effect of extraction temperature and pH (extraction time 2.5 h and water to seed ratio 75:1) on relative viscosity (Y3) of crude polysaccharides.

Table 6
The optimum conditions of the independent variables corresponding to the responses

Response variables	Extraction temperature (°C)		pH		Extraction time (h)		Water to seed ratio	
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded
Yield Y_1	0.65	69.7	0.80	7.7	0.53	3.0	0.28	79
Purity Y_2	−0.05	64.3	−0.01	6.5	−0.12	2.4	0.13	77
Relative viscosity Y_3	−0.44	58.4	−0.08	6.4	0.22	2.7	0.45	82

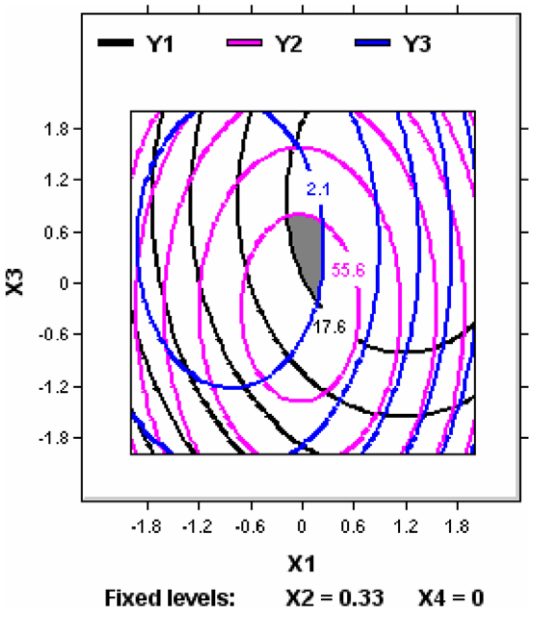

Fig. 4. The optimum region by overlaying contour plots of the three responses evaluated (Y1 – yield, Y2 – purity and Y3 – relative viscosity) as a function of extraction temperature and time.

Table 7
Predicted and experimental values of the responses at optimum conditions

Response variables	Predicted value	Experimental value	
		Mean[a]	Range
Yield (%) Y_1	17.62	17.36 ± 0.63	16.06–17.98
Purity (%) Y_2	56.40	55.81 ± 1.16	53.12–56.87
Relative viscosity Y_3	2.15	2.04 ± 0.30	1.90–2.22

[a] Mean value of five experiments.

3.3. Verification of results

The suitability of the model equations for predicting optimum response values was tested under the conditions: extraction temperature 60 °C, time 2.3 h, pH 7.0 and water to seed ratio 75:1. 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 (Table 7). The experimental and predicted values were found to be not statistically different at 5% level of significance, indicating that the model was adequate for the extraction process. The crude polysaccharides extracted under the optimum conditions were further analyzed for chemical and monosaccharide compositions as shown in Tables 8 and 9, respectively. The crude polysaccharides contained 58% carbohydrates (including uronic acids), 20% proteins, 9% moisture and 4.5% ash. The neutral monosaccharides included 22.6% glucose, 10.0% rhamnose, 7.9% arabinose, 5.0% galactose, and trace amount of xylose (0.8%). The uronic acid in the crude polysaccharides was identified as galacturonic acid (11.8%), which is in agreement with the colorimetric method for uronic acid analysis.

4. Conclusion and future research

The extraction conditions have significant effects on the yield, purity and relative viscosity of crude polysaccharides. Using the contour plots in the response surface methodology, the optimum set of the independent variables was obtained graphically in order to obtain the desired levels of crude polysaccharides extraction. Optimum conditions

Table 8
Chemical analysis of the crude polysaccharides from boat-fruited sterculia seed

Main components	Moisture	Ash	Protein	Total sugar	Uronic acid
Content (wt%)	9.15 ± 0.43[a]	4.47 ± 0.27	20.66 ± 0.61	58.19 ± 0.68	10.65 ± 0.42

[a] Means ± SD (SD = standard deviation; $n = 3$, number of replicates).

Table 9
Monosaccharide composition of the crude polysaccharides

Monosaccharide	Rhamnose	Arabinose	Xylose	Glucose	Galactose	Galacturonic acid
Content (wt%)	10.00 ± 0.30[a]	7.88 ± 0.21	0.77 ± 0.12	22.65 ± 0.35	5.04 ± 0.20	11.85 ± 0.32
Molecular ratio	6.10	5.25	0.51	12.58	2.8	6.11

[a] Means ± SD (SD = standard deviation; $n = 3$, number of replicates).

(temperature 60–65 °C, time 2.3–3.1 h, pH at 7.0 and water to seed ratio at 75:1) for the extraction procedure of crude polysaccharides from boat-fruited sterculia seeds were identified. However, the crude polysaccharides contained significant amount of non-carbohydrate materials, such as proteins and pigments. Further purification and fractionation of the crude polysaccharides are undergoing in order to elucidate the structure and function of polysaccharides from boat-fruited sterculia seed. It is also necessary to establish the structure-bioactivity relationship of the well-known herb in order to expand their application.

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