academicJournals

Vol. 10(7), pp. 47-58, November 2016
DOI: 10.5897/AJBR2015.0862
Article Number: 23BD8B461518
ISSN 1996-0778

http://www.academicjournals.org/AJBR

African Journal of Biochemistry Research

Full Length Research Paper

Extraction, optimization and characterization of crude polysaccharides from *Artemesia Mongolica*

Yahaya Kudush Kawa[1,2]*, Quinck Shang[2], Shangyi Tian[2], John Paul Kaisam[3] and Victoria Kabia[1]

[1]Department of Chemistry Njala University, Sierra Leone.
[2]College of Chemistry, Northeast Normal University, Changchun, China.
[3]Institute of Geographical Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, China.

Received 30 August, 2015; Accepted 8 January, 2016

In this study, the extraction conditions of polysaccharides of *Artemisia mongolia* (PAM) were optimized and their molecular weight, weight distribution, composition and antioxidant activities of the polysaccharides investigated. The degree of polymerization of fructan-oligosaccharides 2-8 was separated by thin layer chromatography (TLC) with good results. Polysaccharides were extracted from the leaves of *A. mongolia* and the effects of the extraction parameters (extraction temperature, extraction time and water to raw material ratio) were optimized under optimal extraction combinations. Based on the study, the maximum yield of polysaccharides (17.38%) was obtained at an extraction temperature of 76.90°C, extraction time of 1.33 h and water-to-raw material ratio of 4.00 ml/mg. The yield of polysaccharides was largely dependent on the extraction parameters. Four isolated fractions of the polysaccharides were further characterized using high-performance liquid chromatography. Using TLC analysis, the degree of polymerization of fructan-oligosaccharides 2-8 was successfully separated. High *A. mongolia* antioxidant polysaccharide with antioxidant activity of 351.3 sc/g was obtained at optimized incubation temperature of 70.37°C, extraction time of 1.45 h and water-to-raw material ratio of 2 ml/mg.

Key words: *Artemisia Mongolia*, crude polysaccharide, antioxidant activity, response surface methodology, box-Behnken design

INTRODUCTION

Artemisia plant, known locally in Chinese Mandarin Language as “mengguhao” and scientifically as *Artemisia mongolica*, was used in this study. *A. mongolica* leaf is a traditional medicinal herb in China with an early history dating back to the “Nei Meng Gu Zhong Cao Yao” era. The genus Artemisia belongs to the family composite

*Corresponding author. Email: yahaya_kawa@yahoo.com, ykawa@njala.edu.sl. Tel: +23279544698.

Arteraceae, which is mainly distributed in the northeast grasslands of China, Korea, Japan, Mongolia and Russia. The leaves of *A. mongolica* are commonly used in folk medicine for the treatment of many diseases, including fever, sore throat, tonsillitis, headache, wounds and hepatitis (Moerman, 1998; Hong et al., 2004).

A number of studies have been conducted on *Artemisia* polysaccharides. Xie et al. (2008) worked on fractionation and characterization of biologically active *Artemisia tripartite* polysaccharides, where five monosaccharides (xylose, glucose, arabinose, galactose and galactosamine) were also isolated. Zhang et al. (2011) screened the chemical characterization of Artemisia seed polysaccharides, with specific emphasis on the analysis of monosaccharide components of *Artemisia* seed polysaccharides (ASP). In the study, gas chromatography (GC) was used to determine the monosaccharide components, atomic force microscopy to capture monosaccharide images and scattering method was used to obtain molecular weight.

Polysaccharides are polymeric carbohydrate structures formed by repeated units joined together by glycosidic bonds. Polysaccharides are widely investigated because of their chemical properties and biological activities (Sun et al., 2010; Wang et al., 2009). Particularly, polysaccharides of traditional medicinal herbs have medical applications and are reported to possess a wide range of pharmacological properties such as anti-tumour, anti-oxidant, anti-diabetic and adjuvant activities. To practicalize the promising applications of polysaccharides, there is the need to study the bio-functional properties of polysaccharides from *A. Mongolica* leaves**.** However, little effort has been devoted to the extraction of *A. mongolica*-based polysaccharides.

The hot-water reflux extraction technique (requiring long extraction time and high temperature) is the most common method used to extract plant-based polysaccharides. However, this extraction method has always had a low efficiency. To obtain high yields of plant-based crude polysaccharides, the extraction process must be optimized by mathematical models (Zhong and Wang, 2010). A good example of such model is the response surface model (RSM), which is based on the Box-Behnken Design (BBD). Additionally, the use of 96-well microplate technique in conjunction with existing chemical methods can efficiently determine the total polysaccharides of a large number of plant samples. This technique not only saves reagents, but also time and sample materials. The microplate-based method of determination of carbohydrates that uses phenol-tetraoxosulphate (VI) acid procedure has also been reported by Masuoka et al. (2006). Then, Tian et al. (2011) noted that the microplate-based method was a more convenient way of optimizing the extraction processes of crude polysaccharides from *A. mongolica* plant. A number of merits make this method preferable over others, including cheap costs and low reagent/ material use.

Thus far, there is little application of BBD and antioxidant of Artemisia plant in the investigation of 96-well format. Thus, BBD was used to optimize the process parameter ratios of water to raw-material, extraction temperature and extraction time of crude polysaccharides. Then, the antioxidant properties of the crude polysaccharides were evaluated and assayed in terms of antioxidant activities by testing the scavenging abilities on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals.

MATERIALS AND METHODS

Instruments and reagents

The leaves of *A. mongolica* were collected in March 2011 from Songnen grassland in Jilin Province, China, and identified by Professor Yifei Yang of Northeast Normal University, Changchun, China. The raw samples were rinsed in distilled water to remove impurities such as dust. The leaves were immediately separated from the plant and the former lyophilized and milled. The derived powder was sieved (through 60 mm mesh screen) and stored at 4°C until use. Inulin, purchased from Sigma Chemical Co. (China, Hongkong) as standard, was dissolved in pure water (10 mg/ml as stock solution). The pure water was obtained from Milli-Q Academic A_{10} water purification system (Millipore Corporation, USA) and EDTA-Ca and TFA purchased from Beijing Shiji. Also, a 20 × 20 cm silica-gel coated glass plate of 250 µm depth (SI 250 JT) was used in the study. The other materials and their origins used in the study included Baker Phillipsburg from NY USA, HP-1050 from US, SHIMADZU-RID-10A HPLC from JAPAN and SHIMADZU UV-2201 from JAPAN.

Absorbance measurement was done in flat-bottomed 96-well format (Thermo Life Sciences, Hampshire, UK) and EDP-plus™ micropipette (Oakland, CA, USA). Then a 25, 250 and 1000 µl liquid-handling robotics was used to dispense solution into the 96-well format. For accuracy and precision, they pipette were respectively 0.3 and 1% or better. All chemicals used in the experiment were of analytical grade and were purchased from Sigma-Aldrich (Malaysia).

Extraction procedure

To remove any color-masking substances, the *A. mongolia* leaves (200 g) were added into ethanol (400 ml) of 80°C water bath for 2.5 h. After oven-drying at 60°C, each pretreated sample was extracted by water at designed extraction temperature, extraction time and water-to-raw-material ratio. The water-extracted solutions were separated from insoluble residues by centrifugation (10,000 rpm for 25 min) and then precipitated by the addition of ethanol. The precipitate was filtered and oven-dried at 60°C for 12 h. The dried crude polysaccharides were refluxed three times with acetone and chloroform to remove lipids. The resultant product was extracted in hot water and then filtered, and the combined filtrate precipitated using ethanol again. The content of the polysaccharides was measured by using the phenol-sulfuric acid method (Dubois et al., 1956).

Analysis of samples

This procedure used to analyze samples in this study was based on

the method proposed by Tian et al. (2011), but with some modifications as follows: 150 µl of concentrated sulphuric acid were added to each well of 96- plate, pre-loaded with 20 µl standard solution (100 mg l^{-1}) — manufacturer's sample solution and blank. 60 µl of 6% aqueous phenol (w/w) were then added to each 96-well plate before incubation at 97°C in an oven for 10 min. Subsequently, the absorbance was measured at 490 nm in a microplate multi-scan reader- measurements were made in triplicates. The response was compared to a inulin-based standard curve and the soluble fructans content expressed as mg l^{-1} of inulin. The purity (%) of fructans was calculated as the sugar content of extraction divided by the weight of dried plant materials.

Polysaccharide extraction and temperature

The test tube was respectively labeled 10, 20, 40 and 60°C and each was repeated three times. It was then placed in a test tube holder and *A. mongolica* leaves weighed by analytical beam balance. Distilled water was added to 10.02–10.08 mg sample weight of the 10, 20, 40 and 60°C test tubes and immerse in 70°C water-bath box for 30 min. The test tubes were then removed from water box and allowed to cool. Next, 2 ml of distilled water was added to 8 ml of the sample and filled to 10 ml. Then different volumes of distilled water, tetraoxosulphate (VI) acid, H_2SO_4, phenol and standard solution were respectively pipetted into the castor box. Using 20 µl of distilled water as blank, 60 µl of phenol and 150 µl of H_2SO_4 were respectively added to different concentrations of 20 µl standard solution (0.2, 0.4, 0.6, 0.8 and 0.10 ml) and 20 µl sample solution, and stirred and placed in oven for 10 min. The solution was placed in a SPECTRAmax® 190-microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, USA) with scanning monochromator for data collection.

Polysaccharide extraction and time

The test tube was respectively labeled 10, 20, 40 and 60 min and each repeated three times. It was then placed in a test tube holder and *A. mongolica* leaves weighed by analytical beam balance. Distilled water was added to 10.02–10.08 mg sample weight of the 10, 20, 40 and 60 min test tubes and immerse in 70°C water-bath box for 30 min. The test tubes were then removed from water box and allowed to cool. Next, 2 ml of distilled water was added to 8 ml of the sample and filled to 10 ml. Then, different volumes of distilled water, tetraoxosulphate (VI) acid, H_2SO_4, phenol and standard solution were respectively pipetted into the castor box. Using 20 µl of distilled water as blank, 60 µl of phenol and 150 µl of H_2SO_4 were respectively added to different concentrations of 20 µl standard solution (0.2, 0.4, 0.6, 0.8 and 0.10 ml) and 20 µl sample solution, and stirred and placed in oven for 10 min. The solution was placed in a SPECTRAmax® 190-microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, USA) with scanning mono-chromator for data collection.

R**aw material-to-water extraction ratio**

The test tube was respectively labeled 10, 20, 40 and 60% and each repeated three times. It was then placed in a test tube holder and *A. mongolica* leaves weighed by analytical beam balance. Distilled water was added to 10.02–10.08 mg sample weight of the 10, 20, 40 and 60% test tubes and immerse in 70°C water-bath box for 30 min. The test tubes were then removed from water box and allowed to cool. Next, 2 ml of distilled water was added to 8 ml of the sample and filled to 10 ml. Then, different volumes of distilled water, tetraoxosulphate (VI) acid H_2SO_4, phenol and standard solution were respectively pipetted into the castor box. Using 20 µl of distilled water as blank, 60 µl of phenol and 150 µl of H_2SO_4 were respectively added to different concentrations of 20 µl standard solution (0.2, 0.4, 0.6, 0.8 and 0.10 ml) and 20 µl sample solution, and stirred and placed in oven for 10 min. The solution was placed in a SPECTRAmax® 190-microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale, USA) with scanning monochromator for data collection.

Experimental design and RSM optimization

Based on single-factor polysaccharides production, the extraction temperature, proper ranges of extraction time and water-to-raw-material ratio were determined. A 3-level, 3-variable BBD SAS of SAS Institute, Cary, NC, USA (Aslan and Cebeci, 2007) was used to determine the best combination of extraction variables for the production of polysaccharides. Based on the single-factor experiment, the variables considered were extraction temperature, extraction time and water-to-raw-material ratio. The independent and dependent variables used in the design are listed in Table 1. Then, Table 2 gives the definitions and coding levels used to develop the model. Each experiment was repeated three times and the average extraction yield of the polysaccharides was taken as the final response.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=1+1}^{k} \beta_{ij} X_i X_j \quad (1)$$

$$X_i = \frac{X_i - X_0}{\Delta X} \quad i = 1, 2, 3 \quad (2)$$

TLC analysis of polysaccharides

The supernatant of the water extract was freeze-dried for 72 h and removed from the lyophilizing machine. Crystal polysaccharides were put into Petri dishes and placed in desiccators. Then, 20 mg weight of the substance was put into test tube and 2 ml distilled water was added to it for TLC analysis. 0.5, 1.2 and 20 µl of the substance were drawn for fructan-oligosaccharide determination. Schleicher and Schuell F-1500 ready foils, developed three times in 1-butanol, 2-propanol and water, were used as mobile phase in water in of ratio of 2:8:40. After three times development, it was dried and placed in oven for 10 min to visualize the different levels of oligosaccharides. The position of sucrose and fructans was ascertained by using urea-phosphoric acid reagent (Wise et al., 1955). Then, using sucrose fructose maltose, etc. as the standard, the polysaccharides were analyzed by paper chromatography (PC) as reported by Santoiani et al. (1993).

Antioxidant activity determination

DPPH-free radical scavenging activity of each sample was determined as described by Liu et al. (2009). The extract was reconstituted with distilled water and pre-diluted 20 times. Aliquots of each sample (1 ml) were added to 3 ml of methanolic DPPH solutions (0.1 mM). Discolorations were measured at 516.3 nm after incubation for 30 min at 30°C in the dark.

$$\%DPPH_{sc/g}\ extract = \{(A_{count} - A_{sample}) / A_{count}\} \times 100/W_{EAP} \quad (3)$$

Table 1. BBD results for observed and predicted values of yield of *Artemisia mongolica* polysaccharide (%) and %DPPHsc/g extract.

Run	Coded variables			Observed values (Y_1)	
	X_1 (Temperature)	X_2 (Time)	X_3 (Ratio)	Yield (%)	%DPPHsc/g extract
1	70	80	40	9.43	171.24
2	90	80	40	7.07	148.68
3	70	160	40	9.70	143.34
4	90	160	40	17.38	104.28
5	70	120	20	4.57	351.30
6	90	120	20	3.47	287.28
7	70	120	60	11.86	107.22
8	90	120	60	9.21	91.74
9	80	80	20	6.23	331.08
10	80	160	20	10.76	288.24
11	80	80	60	16.02	104.82
12	80	160	60	14.37	99.72
13	80	120	40	8.70	150.48
14	80	120	40	8.21	149.46
15	80	120	40	8.71	149.34
16	80	120	40	8.31	151.92
17	80	120	40	8.11	162.18

RESULTS AND DISCUSSION

Phenol-tetraoxosulphate (VI) acid/sulphuric acid procedure in 96-well format

About 150 µl of concentrated tetraoxosulphate (VI) acid were added to each 96-well format containing 20 µl of standard solution (1000 mg/l), manufacturer's sample solution and the blank. Then 60 µl of 6% aqueous phenol (w/w) were added to each well of micro-plate before incubation at 80°C in oven for 10 min. Subsequently, the absorbance was immediately read at 490 nm in a micro-plate multi-scan reader. Also, all measurements were done in triplicate. The response was compared with the inulin-based standard curve and the soluble polysaccharide content expressed in g/l of inulin. The purity (99%) of polysaccharides was calculated as the sugar content of extraction divided by the weight of dried materials as plotted in Figure 1.

Effect of extraction temperature on polysaccharide yield

Different extraction temperatures set respectively at 50, 60, 70, 80 and 90°C were used to investigate the effect of temperature on the extraction of crude polysaccharides from *A. mongolica* while all the other reaction conditions were held constant (water-to-raw-material ratio of 40 and extraction time of 2.5 h). Figure 2 shows that the maximum extraction yield of crude polysaccharides in terms of temperature is 70-90°C. There was no increase in yield with further increase in extraction temperature beyond 90°C. Thus 70-90°C was adopted as the optimal extraction temperature in this experiment.

Effect of extraction time on polysaccharide yield

The effect of extraction time on the extraction yield of *A. mongolica* polysaccharides is shown in Figure 3. In the first step, the extraction time was set respectively at 80, 100, 120, 140 and 160 min while other extraction parameters were held constant (water-to-raw-material of 40 and extraction time of 70°C). It was noted that the extraction yield increased with increasing extraction time (80-120 min), with the peak yield occurring at 120 min. For times longer than 120 min, no further increase was noted in *A. mongolica* polysaccharide extraction yield.

Effect of water-to-raw-material ration on polysaccharide yield

The effect of different ratios of water-to-raw-material (10, 20, 40 and 60) on the extraction yield *A. mongolica* polysaccharides is shown in Figure 4. For the plot (Figure 4), the other extraction factors (extraction temperature and extraction time) were held respectively at 70°C and 30 min.

Table 2. List of ANOVA results for response surface models, including estimated regression model for the relationship between response variables (yield and %DPPHsc/g) and independent variables (X_1, X_2, X_3).

Source	Sum of squares	DF	Mean square	F-value	p-value
Yield (%)[a]					
Model	153.23	9	17.03	260.46	<0.0001
X_1	6.00	1	6.00	91.85	<0.0001
X_2	3.06	1	3.06	46.78	0.0002
X_3	78.26	1	87.26	1334.93	<0.0001
X_1^2	18.63	1	18.63	284.95	<0.0001
X_2^2	25.56	1	25.56	391.05	<0.0001
X_3^2	4.02	1	4.02	61.44	0.0001
X_1X_2	0.58	1	0.58	8.94	0.0202
X_1X_3	0.60	1	0.60	9.15	0.0192
X_2X_3	9.51	1	9.51	145.45	<0.0001
Residual	0.46	7	0.065		
Lack of fit	0.14	3	0.047	0.59	0.6544
Pure error	0.32	4	0.079		
Total	153.68	16			
%DPPHsc/g extract (%/g)[c]					
Model	1.120E+005	9	12447.26	282.48	<0.0001
X_1	2489.36	1	2489.36	56.49	0.0001
X_2	1801.80	1	1801.80	40.89	0.0004
X_3	91288.37	1	91288.37	2071.75	<0.0001
X_1^2	57.89	1	57.89	1.31	0.2894
X_2^2	211.24	1	211.24	4.79	0.0647
X_3^2	15369.32	1	15369.32	348.80	<0.0001
X_1X_2	68.06	1	68.06	1.54	0.2539
X_1X_3	589.03		589.03	13.37	0.0081
X_2X_3	352.69		352.69	8.00	0.0254
Residual	308.44	7	44.06		
Lack of Fit	191.25	3	63.75	2.18	0.2335
Pure Error	117.19	4	29.30		
Total	1.123E+005	16			

From Figure 4, it is clear that the extraction yield of *A. mongolica* polysaccharides increased sharply and peaked at 5.26% for an extraction ratio of 4 ml/mg. Thereafter, the extraction yield of *A. mongolica* polysaccharides decreased after the water-to-raw-material ratio exceeded 4 ml/mg (Figure 4).

BBB response surface analysis

The extraction yields of *A. mongolica* polysaccharide**s** were investigated in the study. The parameters were chosen after preliminary analysis with the highest yield of polysaccharides at desired antioxidant activity. The results of 17 runs of BBD in Table 2 include the design and observed responses. There was a close agreement between field-observed and predicted values. The maximum yield (17.38%) was noted under the experimental conditions of X_1 = 76.90 °C, X_2 = 1.33 h and X_3 = 4 ml/mg. On the other hand, the range of antioxidant property (%DPPHsc/g extract) was 91.74–351.32 %/g. The highest %DPPHsc/g extract (351.32 %/g) was observed under the experimental conditions of X_1 = 70.37°C, X_2 = 1.45 h and X_3 = 2 ml/mg. Note that the conditions changed with required responses.

Model fit

Table 3 presents the results of the model fits (quadratic

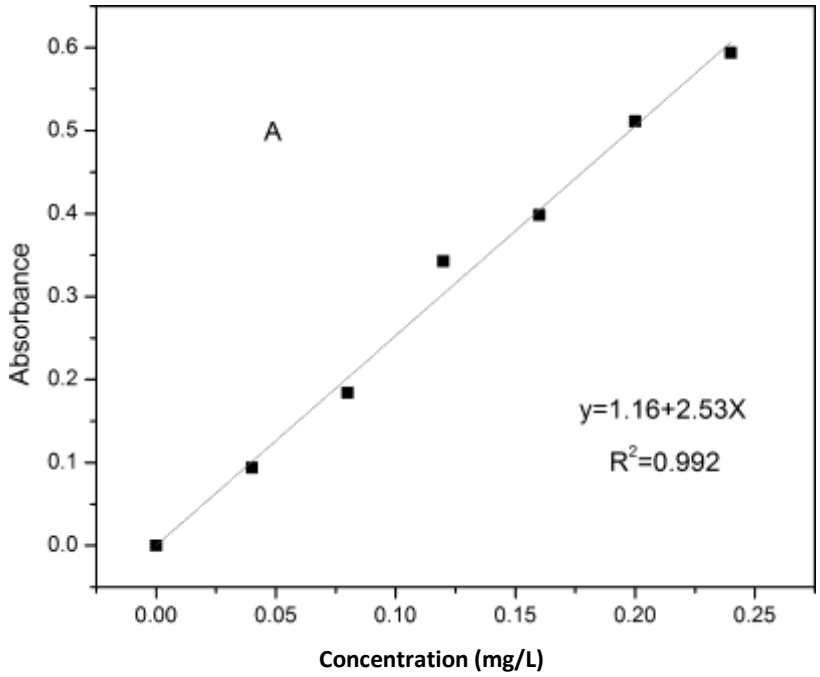

Figure 1. *Artemisia mongolica* polysaccharide calculation based on inulin-based standard curves and the relative absorbances.

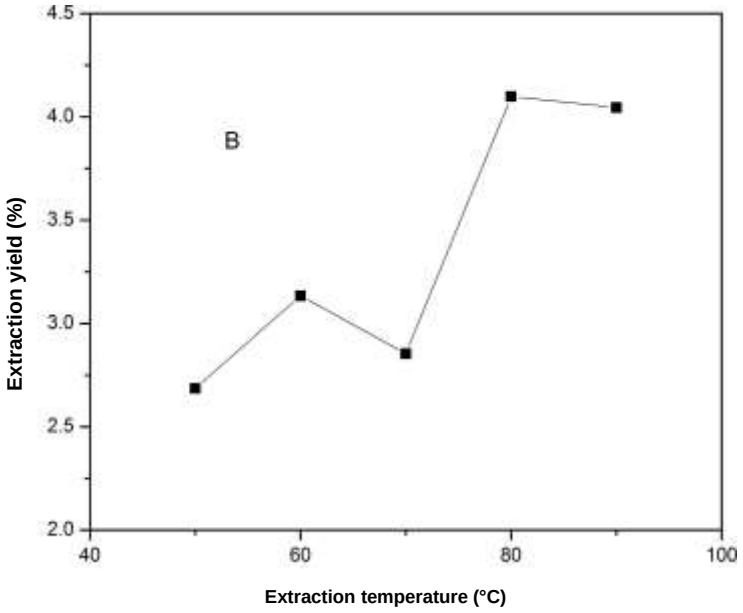

Figure 2. Effect of temperature on extraction yield of *Artemisia mongolica* polysaccharides.

and linear) to observed data. The results of analysis of variance (ANOVA) suggest that the quadratic model significantly explained the responses of the extraction yields and antioxidant activities. The fitted quadratic models for extraction yield and %DPPHsc/g extract in the coded variables are quantified respectively in Equations 4

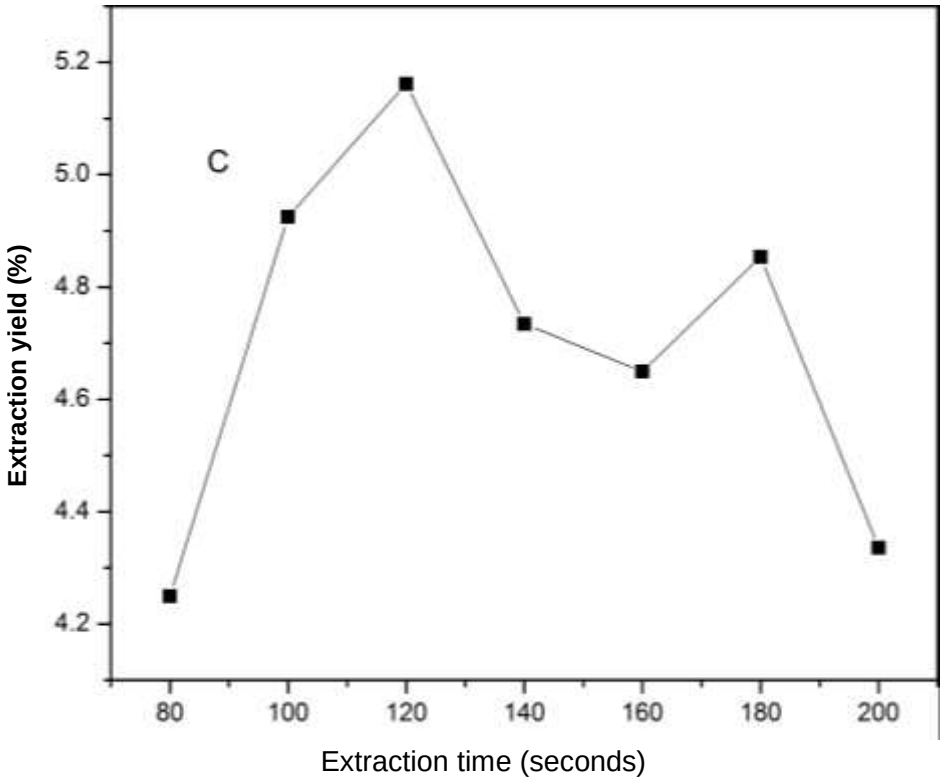

Figure 3. Effect of time on extraction yield of *Artemisia mongolica* polysaccharides.

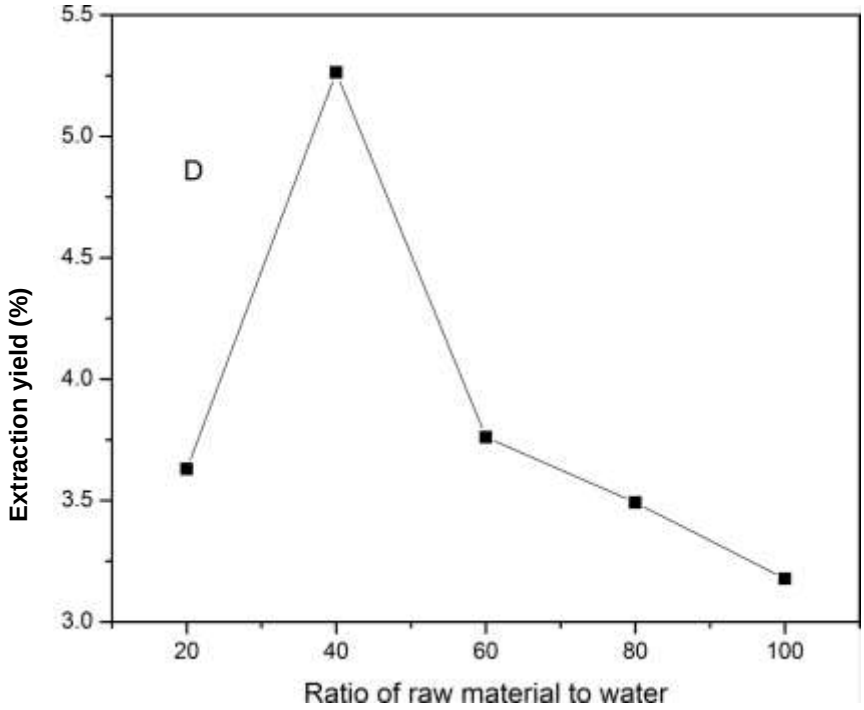

Figure 4. Effect of water-to-raw-material ratio on extraction yield of *Artemisia mongolica* polysaccharides.

Table 3. A list of fit statistics for dependent variable Y.

Y variable		Master model	Predictive model
Yield	RMSE	9.03	9.03
	R-square	99.70	99.70
	Adjusted R-square	99.32	99.32
	Coefficient of variation	2.83	2.83
%DPPHsc/g	RMSE	176.03	176.03
	R-square	99.73	99.73
	Adjusted R-square	99.37	99.37
	Coefficient of variation	3.77	3.77

Table 4. Fit statistics of Y.

Y variable		Master model	Predictive model
Yield	RMSE	9.03	9.03
	R-square	99.70	99.70
	Adjusted R-square	99.32	99.32
	Coefficient of variation	2.83	2.83
%DPPHsc/g	RMSE	176.03	176.03
	R-square	99.73	99.73
	Adjusted R-square	99.37	99.37
	Coefficient of variation	3.77	3.77

and 5. The significance of each coefficient was determined using the F-test and p-value in Table 4. The corresponding variables can be more significant if the absolute F-value increases greater and the p-value decreases (Atkinson and Donev, 1992):

$$EY = 8.40 - 0.87x_1 + 0.62x_2 + 3.3x_3 + 0.38x_1^2 - 0.39fx_2^2 - 1.54x_3^2 - 2.1x_1x_2 + 2.46x_1x_3 - 0.98x_2x_3 \quad (4)$$

$$AP = 152.68 - 17.64x_1 - 15.010.62x_2 - 106.82x_3 - 3.71x_1^2 - 7.08x_2^2 + 60.42x_3^2 - 4.13x_1x_2 + 12.14x_1x_3 + 9.39x_2x_3 \quad (5)$$

where EY is extraction yield and AP antioxidant property

$$\left(\%\frac{\text{DPPHsc}}{\text{g extract}}\right)$$

Extraction yield: The term with the largest effect on polysaccharide extraction yield was a linear (X_1, X_2, X_3), followed by quadratic (X_1X_2, X_1X_3, X_2X_3) and then the interaction (X_1X_2, X_1X_3, X_2X_3) terms (Table 3). The results in Table 3 suggested that only the changes in extraction temperature, extraction time and in water-to-raw material ratio had significant effects ($p < 0.0001$) on the yield of extracted polysaccharides. The coefficient of determination (R^2) of the model predicted responses was 0.9970 with p-value of 0.59. These values gave a relatively good fit to the mathematical model in Equation 4.

%DPPHsc/g extract antioxidant activity: In terms of antioxidant activity, linear (X1, X2, X3) and quadratic (X1, X2) terms of polysaccharide extraction parameters had the largest effect ($p < 0.0001$), followed by the interaction (X1X3 and X2X3) terms. However, quadratic (X3) and the interaction terms of the extraction parameters were not significant ($p > 0.05$). The coefficient of determination (R^2) of the model predicted response was 0.9972 with p-value of 2.29. This suggested that there was an excellent fit to the mathematical model in Equation 5. Thus, the responses were sufficiently explained by the models.

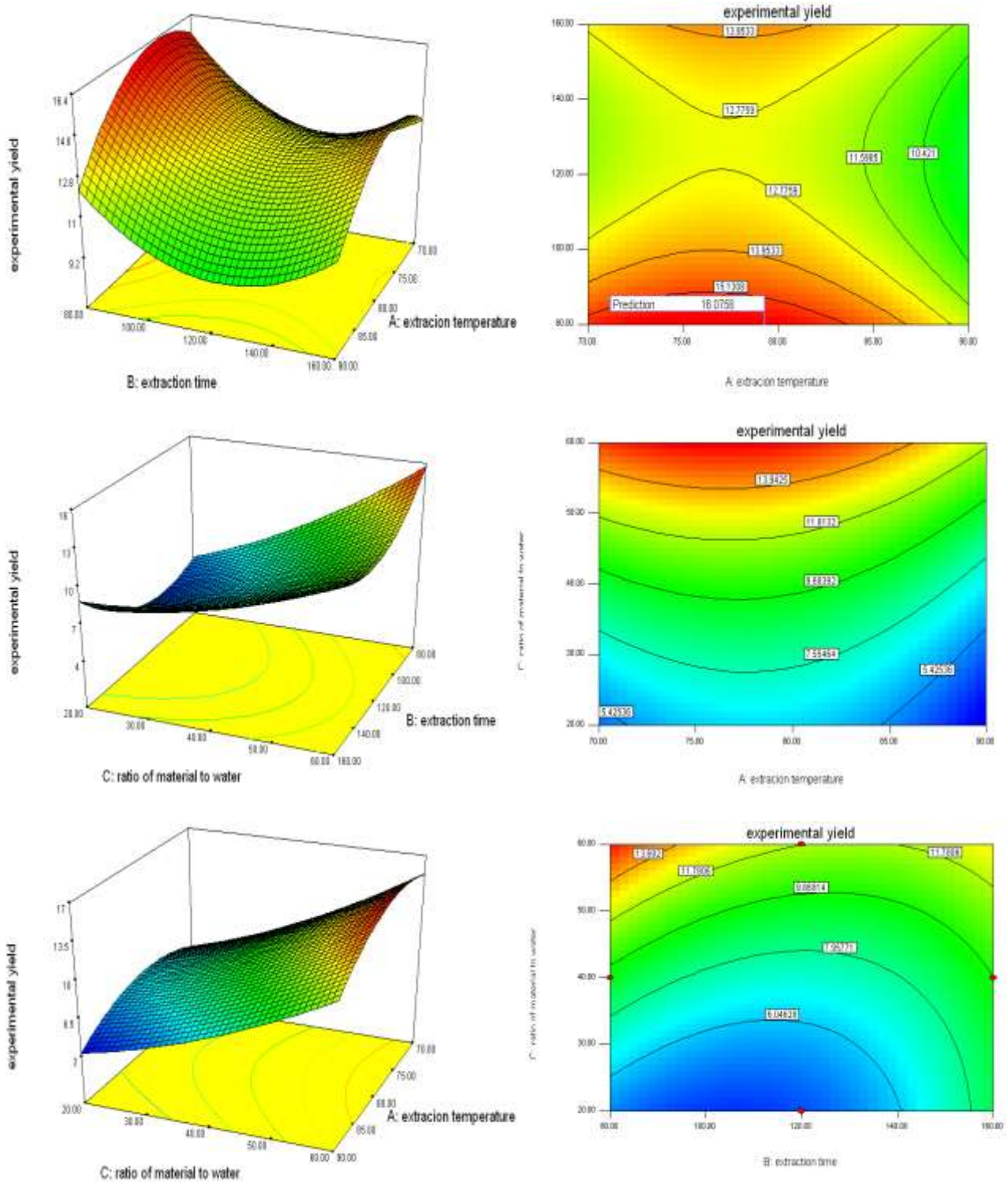

Figure 5. Three-dimensional response surfaces and contours of extraction yields of *Artemisia mongolia* polysaccharides.

Response surface model and contour plot

Extraction yield explanation: Three-dimensional (3D) and contour plots of the polysaccharide extraction yields are given in Figure 5. The result in Table 3 showed that all the extraction parameters significantly ($p < 0.05$) or

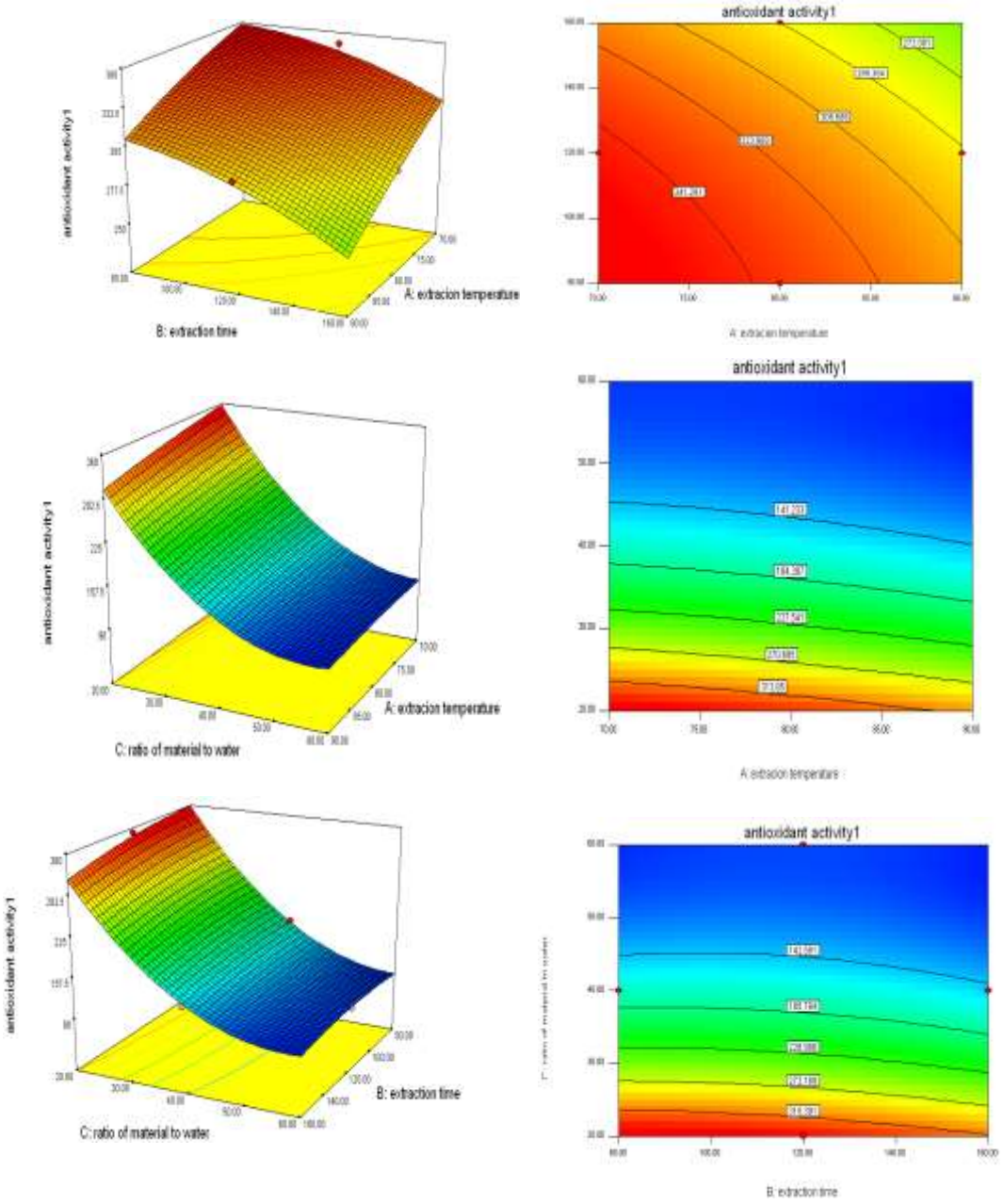

Figure 6. Three-dimensional response surfaces and contours of antioxidant activities (%DPPHsc/g extract) of *Artemisia mongolia* polysaccharides.

highly significantly ($p < 0.0001$) contributed to the extraction response. The maximum yield of the polysaccharides (17.38%) was obtained at an extraction temperature of 76.90°C, extraction time of 1.33 h and water-to-material ratio of 4 ml/mg.

Antioxidant activity (%DPPHs/g extract)

The 3D response surfaces and contours of %DPPHsc/g extract are given in Figure 6. It was apparent that the polysaccharides possessed antioxidant activity by scavenging DPPH free radicals. It was also observed that with 65–70°C extraction temperature or 80–160 min extraction time, there was increased antioxidant activity. Extract of water-to-raw material ratio of 2.5 or less seemed to have higher antioxidant activity when compared with water-to-raw material ratio higher than 3.0. At extraction temperature of 70.37°C (Figure 6a) and extraction time of 160 min (Figure 6b), %DPPHsc/g

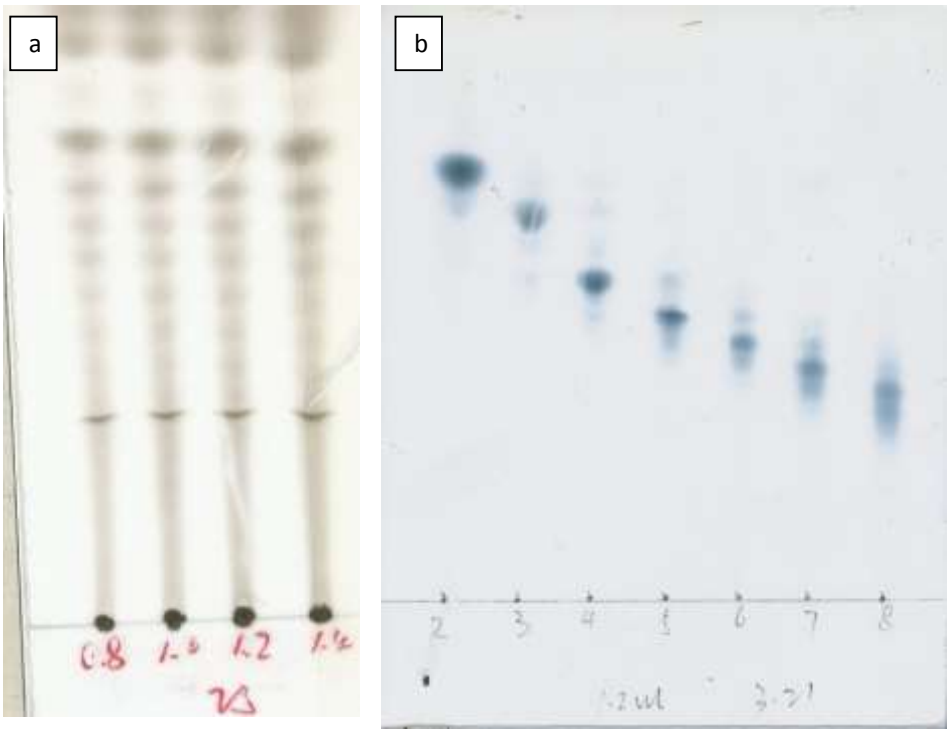

Figure 7. Plots of fructan-oligosaccharide in *Artemisia mongolica* plant 2-8 (a), depicting the mobilities of fructan-oligosaccharides DP 2–8 degree of polymerization (b). Note that each contains fructan equivalent in ethanol sample value.

extract reached 351.3.

Predictive model verification

Based on the above findings, an optimization analysis was done to evaluate the optimal operating conditions for the extraction of high yields of polysaccharides and antioxidant activities. Table 4 has two optimum conditions based on the combination of all the responses. These optimal conditions were: 1) extraction temperature of 76.90°C, extraction time of 1.33 h and water-to-material ratio of 4 ml/mg; and 2) extraction temperature of 70.37°C, extraction time of 1.45 h and water-to-raw material ratio of 2 ml/mg. The corresponding optimum condition of the polysaccharides was 16.02% and that of %DPPHsc/g extract was 351.3%. Only small deviations were noted between the actual and predicted values. Thus, the model was applicable in optimizing the processes of *A. mongolica* polysaccharides extraction.

TLC analyses

The purification of fructan-oligosaccharide poly-saccharides with high molecular weight was qualitatively assessed at each stage of the protocol after ascending thin layer chromatography. The extracted samples were applied to silica-gel coated origin 20 × 20 cm glass plate. Sample were developed in triplicate in butan-1-ol/pro-2-ol/water of 3:12:4 ratio (V/V/V) at room temperature. Then qualitative analysis of fructo-oligosaccharides by TLC method (Figure 7) showed the existence of low DP members of EDTA-Ca series (DP 2-
8) in the fractions, although changes in the relative proportions varied with treatment. For plants watered every 30 days, fructose, sucrose and other components of the series were more concentrated; reflecting the observed increase in total fructose in the oligosaccharide fraction. This increase occurred simultaneously with the reductions in polysaccharide fractions in Figures 7a and b.

The purification of fructan-oligosaccharide poly-saccharides with high molecular weight was qualitatively assessed at each stage of the process after ascending TLC extracted samples were applied to silica-gel coated origin 20 × 20 cm glass plate. The samples were developed in triplicate in butan-1-ol/pro-2-ol/water with ratio of 3:12:4 (v/v/v) at room temperature. TLC-based qualitative analysis of fructo-oligosaccharides (Figure 8) showed the existence of low DP members of the EDTA-Ca series (DP 2- 8) in this fractions, although changes in

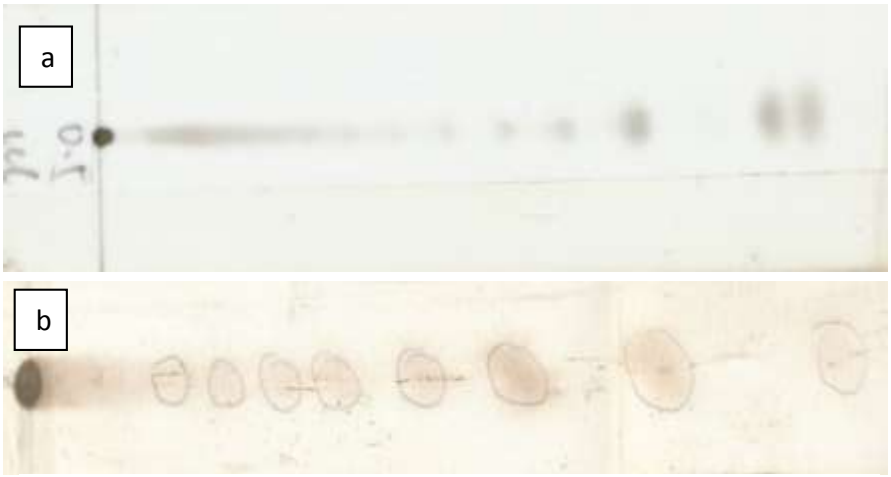

Figure 8. Plots of fructan-oligosaccharide in *Artemisia mongolica* plant 2–8 (a), with mobilities of fructan-oligosaccharides of DP 2–8 degree of polymerization (b).

the relative proportions varied with treatment. In plants watered every 30 days, fructose, sucrose and the other components of the series were more concentrated, reflecting the observed increase in total fructose in the oligosaccharide fractions. This increase occurred simultaneously with reduction in polysaccharide fractions in Figure 8a and b.

Conclusions

The single-factor experiments and BBD together with RSM simulations were used to determine the optimum process parameters with high extraction yield and antioxidant activity of *Artemisia mongolica* polysaccharides. Based on ANOVA analysis, the effects of extraction temperature, extraction time and extraction water-to-material ratio were significant. Quadratic models were fitted to the responses of extraction yield and antioxidant activity. Two optimal conditions were determined: 1) extraction temperature of 76.90°C, extraction time of 1.33 h and extraction water-to-raw material ratio of 4 mL/mg; and 2) extraction temperature of 70.37°C, extraction time of 1.45 h and water-to-raw material ratio of 2 mL/mg. This optimum condition for the polysaccharides was 17.38% and that for %DPPHsc/g extract was 351.3%. Thus, the model was applicable in the optimization of the processes of *A. mongolica* polysaccharide extraction. Preliminary identification of the polysaccharides showed the potential for the use of antioxidant in medicine or health-care foods. The purification of fructan-oligosaccharide polysaccharides was possible after ascending TLC and triple plate development by DP 2-8 fructan-oligosaccharide analyses.

Conflict of interests

The authors have not declared any conflict of interests.

REFERENCES

Aslan N, Cebeci Y (2007). Application of Box-Behnken design and response surface methodology for modeling of some Turkish coals. Fuel 86:90-97.

Atkinson AC, Donev AN (1992). Optimum experimental designs. Oxford: Oxford University Press. pp. 132-189.

Dubois M, Gillis KA, Hamilton JK, Rebers PA, Smith F (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28(3):350-356.

Hong KJ, Dunn DM, Shen CL, Pence BCE (2004). Effects of Ganoderma lucidum on apoptotic and anti-inflammatory function in HT-29 human colonic carcinoma cells. Phytother Res.18:768-70.

Liu SC, Lin JT, Wang CK, Chen HY, Yang DJ (2009). Antioxidant properties of various solvent extracts from lychee (Litchi chinenesis Sonn.) flowers. Food Chem.pp. 577-581.

Moerman DE (1998). Native American ethnobotany, Timber Press, Portland.

Santoiani CS, Tognetti JA, Pontis HG, Salerno GL (1993). Sucrose and fructan metabolism in wheat roots at chilling temperatures. Physiol. Plant. 87(1):84-88.

Sun Y, Li T, Yan T, Liu J (2010). Technology optimization for polysaccharides (POP) extraction from the fruiting bodies of *Pleurotus ostreatus* by Box–Behnken statistical design. Carbohydr. Polym. 80:242-247.

Wang L, Huang HY, Wei YY, Li XX, Chen ZX (2009). Characterization and anti-tumor activities of sulfated polysaccharide SRBPS2a obtained from defatted rice bran. Int. J. Bio. Macromol. 45(4):427-431.

Wise CS, Dimler RJ, Davis HA, Rist CE (1955). Determination of easily hydrolyzable fructose units in dextran preparation. Anal. Chem. 27:33-36.

Zhang J, Jia S, Liu Y, Wu S, Ran J (2011). Optimization of enzyme-assisted extraction of the *Lycium barbarum* polysaccharides using response surface methodology. Carbohydr. Polym. 86:1089-1092.

Zhong K, Wang Q (2010). Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. Carbohydr. Polym. 80:19-25.