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Extraction of Polysaccharide from *Dendrobium nobile* Lindl. by Subcritical Water Extraction

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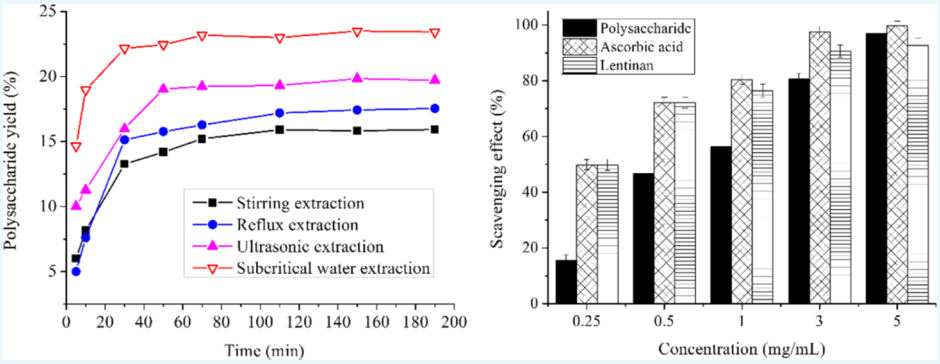
Supporting Information



ABSTRACT: Subcritical water extraction (SWE) uses hot compressed water as an effective solvent for both polar and nonpolar compounds and has been developed as an environmentally benign extraction technology for natural materials. Polysaccharides as one of the main ingredients in *Dendrobium* plants showed obvious biological activity. Thus, SWE of polysaccharides obtained from *Dendrobium nobile* Lindl. was investigated in this work. The response surface methodology (RSM) was combined with a Box−Behnken design to evaluate the influence that the three independent variables had on the response. The optimal extraction conditions (determined via RSM) were 129.83 °C extraction temperature, 16.71 min extraction time, and 1.12 MPa extraction pressure. The maximum predicted polysaccharide yield was 20.67%, which corresponded well with the experiential extraction (21.88%). The polysaccharides obtained from either the stirring extraction, refluxing extraction, ultrasound extraction, or SWE methods were compared, and the extraction processes were modeled. The molecular weight, monosaccharide composition, and antioxidative activities of the polysaccharides were analyzed.

1. INTRODUCTION

Dendrobium nobile Lindl., Orchidaceae, is a herbal plant commonly used in Chinese traditional medicine and it is one of the several *Dendrobium* species that were specified in the Chinese Pharmacopeia.[1,2] Previous research has shown that *Dendrobium* has applications as an agent in antioxidants, and also have anticancer, immunomodulatory, hepatoprotective, and neuroprotective activity.[3] Many compounds have been extracted and isolated from the *Dendrobium* species to better understand these health functions,[4] including polysaccharides,[5,6] alkaloids,[7,8] phenolics,[9,10] phenanthrenes,[11] and bibenzyls.[12,13] Other chemical constituents were detected in *Dendrobium officinale*, such as phenols, acids, esters, and amides.[14] Polysaccharides are considered to be one of the main active components in *Dendrobium* plants,[15] demonstrating antitumor,[16] antiviral,[17] antihyperglycemic, and immunomodulatory activities.[18,19] Moreover, polysaccharides as ideal biodegradable polymer materials can be used as drug carrier, food packaging, pharmaceutical preparations, and so forth.[20,21]

Most of the previous research pertaining to the compounds in the polysaccharides from *D. nobile* Lindl., have focused on their structural characteristics and pharmacological properties. Wang et al. reported that some of the *D. nobile* polysaccharides displayed remarkable immunomodulatory effects. Bioactive tests in vitro revealed that five water-soluble polysaccharides (DNP-W1B, DNP-W2, DNP-W3, DNP-W4, and DNP-W5) could stimulate ConA- and LPS-induced T and B lymphocyte proliferation.[22−26] They also found that *D. nobile* polysaccharides have strong antitumor activities, and two water-soluble polysaccharides (DNP-W1 and DNP-W3) exhibited high antitumor activities against Sarcoma 180 in vivo and HL-60 in vitro. Polysaccharides were extracted thrice by distilled water for 2 h at 80 °C.[27] Li et al. isolated and characterized a neutral polysaccharide (DNPE6(4)) from *D. nobile* Lindl and studied its anti-TMV and anti-CMV activities for the first time in

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vivo.[28] Some others extracted and purified homogeneous heteropolysaccharides which could alleviate vinorelbine-induced decrease of macrophages in vivo[29] and presented significant immune-modulating activities.[30,31] Luo et al. extracted polysaccharides from the stem of the *D. nobile* Lindl. three times with hot water for 2 h each time, where the structure of the polysaccharides were characterized and the antioxidant activities were evaluated in vitro.[32,33] They evaluated the mechanism of the antitumor activities and the immunomodulation effects of the four polysaccharide fractions taken from the *D. nobile* Lindl. in vivo.[34] Pan et al. reported polysaccharides taken from four different *Dendrobium* species were extracted thrice with boiling distilled water, each time for 0.5 h, where their hypoglycemic and antioxidative activities were compared in vivo.[35] Hot water extraction remains the most common extraction method for polysaccharides. This method is typically time-consuming, laborious, and has low selectivity and/or low extraction yields,[36] making it necessary to bring forth novel extraction methods that would circumvent this.

Subcritical water extraction (SWE) uses hot compressed water as an effective solvent for polar and nonpolar compounds, where it is developed as an environmentally benign extraction technology for natural materials.[37,38] Subcritical water is liquid water under pressure, at a temperature range between the usual boiling point (100 °C) and critical temperature (374.1 °C).[39,40] At these conditions, the thermal motion of water molecules increases, thus changing its properties.[41] Cooler water should extract more water-soluble organic compounds, whereas hotter temperature water should extract less soluble organic compounds. The pressure determines the state of the water by changing the density to enhance water solvency.[42] SWE has been used to extract polyphenolic and antioxidant compounds from many natural products,[43−45] although it has not been used as an extraction method for polysaccharides from the *D. nobile* Lindl. In this work, we proposed SWE as a feasible processing method for the extraction for polysaccharides from the *D. nobile* Lindl. The study used the Box−Behnken design (BBD) of response surface methodology (RSM) to optimize the extraction experiments. The polysaccharides were analyzed via UV−vis spectrophotometry. The extraction process was modeled and the polysaccharide's antioxidative activities were analyzed.

2. MATERIALS AND METHODS

2.1. Materials and Chemicals. The dry stem of the *D. nobile* Lindl. was purchased from Sichuan Wanan Industrial Development Co., Ltd. (Chengdu, China). The dry mushroom was obtain from Jiangnan biotechnology Co., Ltd. (Jiangsu, China). Reference standard glucose (GC purity > 99.5%) was purchased from Sigma-Aldrich Co., Ltd. (Beijing, China). The other chemicals were of analytical reagent grade.

2.2. Subcritical Water Extraction. SWE was performed on a stainless steel batch reactor (inner volume 100 mL), constructed in house. A schematic drawing of the SWE is presented in Figure 1. The dry stem of the *D. nobile* Lindl. was ground in a mill until the powder was less than 0.45 mm. The powder material and purified water were placed in the vessel at a solid to liquid ratio of 1:25 (g/mL). The vessel was then sealed and purged with inert N_2. The extraction pressure (0.5−1.5 MPa) was controlled at a predetermined value. The reactor was heated to the experimental temperature (120−160 °C) by an electric heater. The mixture was agitated by a magnetic stirrer at 300 rpm. The pressure and temperature inside the reactor during each experiment were measured by the pressure gauge and the temperature controller. The extraction time was tracked after the interior temperature of the vessel reached the target level. After a predetermined treatment time (5−20 min), the vessel was quickly immersed into an ice bath to stop the extraction. The mixture in the vessel was paper-filtered and stored at 4 °C for further analysis.

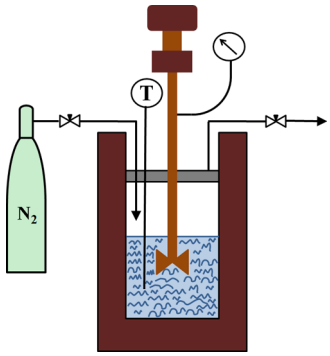

Figure 1. Schematic diagram of SWE.

The mixtures were combined and concentrated under the reduced pressure via a vacuum rotary evaporator at 65 °C. The mixtures were centrifuged at 4000 rpm for 10 min to obtain the supernatant. The supernatant was then precipitated by adding four times the volume of 95% alcohol, where the mixture was left to rest overnight at 4 °C. The precipitate was centrifuged at 4000 rpm for 10 min, washed with absolute ethanol, acetone, and diethyl ether, dissolved in water, and then the procedure was repeated. The crude polysaccharide was obtained via vacuum freeze-drying.

2.3. UV−Vis Spectrophotometry Determination. The phenol−sulfuric acid method was used to test the polysaccharide sample, as it is low cost and UV−vis spectrophotometry is easily available.[46,47] Glucose (0.02−0.08 mg/mL) was used as the reference to obtain a calibration curve. The linear regression equation was: $A_{488} = 9.6639C + 0.0016$ ($r = 0.9976$), where A_{488} was the absorbance at 488 nm and C was the concentration of the glucose sample (mg/mL). One milliliter of the polysaccharide solution (0.20 mg/mL) was mixed thoroughly with 1 mL of the fresh 5% aqueous solution phenol in a test tube, then 5 mL of concentrated sulfuric acid (98%) was rapidly added to the mixture. The samples were vortexed for 30 s, placed in an oil bath, and then heated for 20 min at 100 °C for color development. The reference solutions were prepared via the same methods, although a 1 mL aliquot of the polysaccharide solution replaced the water. The samples were cooled to room temperature in a water bath, the mixtures were measured, and the absorbance was recorded at 488 nm on a UV−vis spectrophotometer (Shanghai Yoke instruments Co., Ltd., Shanghai, China). The total polysaccharide concentration was calculated with glucose as the standard.

2.4. Single Factor Experiments. The extraction temperature, solvent/solid ratio, extraction time, and pressure were the main factors influencing the SWE of the polysaccharides. During the single factor experiments, one factor was changed while the other factors were kept constant in each experiment, and all the experiments were repeated three times.

2.5. Experimental Design of RSM. *2.5.1. Optimization of Process Parameters.* The conditions for the SWE of the

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polysaccharides were further optimized from the *D. nobile* Lindl. The BBD serves as the RSM and was employed using a Design-Expert 7.1.6 Trial (State-Ease, Inc., Minneapolis MN, USA). BBD determined the maximum efficiency for an experiment involving three factors and three levels, the number of experiments conducted was less than a central composite design.[48] The three independent variables, the extraction temperature (X_1), extraction time (X_2), and pressure (X_3), were optimized by the BBD in order to obtain the maximum polysaccharide yield (Y). Each independent variable for X_1, X_2, and X_3 were evaluated at three levels, see Table 1. The

Table 1. Box−Behnken Design of Three Variables with Polysaccharide Yields

	parameters and levels			
run	X_1: temperature (°C)	X_2: time (min)	X_3: pressure (MPa)	polysaccharide yield (%)
1	120	12.5	1.5	16.12
2	140	12.5	1.0	20.11
3	120	20.0	1.0	19.28
4	140	12.5	1.0	19.32
5	140	20.0	1.5	17.24
6	160	20.0	1.0	17.16
7	140	20.0	0.5	9.63
8	160	12.5	1.5	15.34
9	140	5.0	1.5	12.88
10	120	12.5	0.5	11.08
11	120	5.0	1.0	17.95
12	140	5.0	0.5	9.89
13	160	12.5	0.5	7.49
14	160	5.0	1.0	17.56
15	140	12.5	1.0	20.30

experimental design totaled 15 runs, including the three replicates at the center point. All experiments were performed at random. The experiments were performed in duplicates and the average polysaccharide yield was used as the response.

2.5.2. Statistical Analysis. The BBD experimental data were analyzed. Analysis of variance (ANOVA) was performed to determine the adequacy of the developed model and the statistical significance of the regression coefficients.[49] The second-order polynomial coefficients were calculated and analyzed using a Design-Expert 7.1.6 Trial (State-Ease, Inc., Minneapolis MN, USA). Statistical significance was considered at $P < 0.05$.[50]

2.6. Modeling of the Extraction Process. The kinetic model enabled the exploration of the SWE process and predicted the experimental results.[51] A pseudo-first-order kinetic model was employed to fit the experimental data. The rate constant was defined as the slope of the plot for the equation of the first-order reaction[52,53]

$$\ln\left(\frac{C_\infty}{C_\infty - C}\right) = k_{obs}t + a \quad (1)$$

where C_∞ was the equilibrium concentration of the polysaccharide in the solution (g/L) at infinite time ($t = \infty$), C was the concentration of the polysaccharide in the solution (g/L) at time t, k_{obs} was the rate constant (min^{-1}) of the extraction process, and a was the constant for the washing step of the polysaccharide extraction. If the sample was removed after each time interval, the total volume of solution could change. The maximum yield (Y_m) of the given experiments replaced C_∞, where eq 1 becomes[54,55]

$$\ln\left(\frac{Y_m}{Y_m - Y}\right) = k_{obs}t + a \quad (2)$$

Equation 2 was used to fit the experimental data and to obtain Y_m and k values.

The stirring extraction, refluxing extraction, ultrasound extraction, and SWE were used to extract polysaccharides from the *D. nobile* Lindl. A series of experiments *D. nobile* Lindl. (particle sizes < 0.45 mm) were performed. They were prepared by water immersion to obtain a solvent/solid ratio of 1:25 (g/mL), where eight mixtures were extracted for 5, 10, 30, 50, 70, 110, 150, and 190 min. (1) The *D. nobile* Lindl. powder was then put in a conical flask with 60 °C water under magnetic stirring for the stirring reaction. (2) The *D. nobile* Lindl. powder was loaded in a round-bottom flask with water, and then heated to 90 °C in an oil bath under reflux for the refluxing extraction. (3) Ultrasound extraction was performed by placing the *D. nobile* Lindl. powder and water in an ultrasonic cleaner (KQ-300E, Kunshan ultrasonic instrument Co., Ltd., Kunshan, China) with a frequency of 40 KHz and a nominal power of 300 W at 60 °C. (4) The *D. nobile* Lindl. powder and water were placed in a high-pressure batch reactor for the SWE (see the section of 2.2). The operating pressure was 1.0 MPa and extraction temperature was 130 °C. Each sample was paper-filtered, the filtrate was obtained, and then analyzed.

2.7. Analysis of Molecular Weight and Monosaccharide Compositions. The molecular weights of the polysaccharides were determined by the gel permeation chromatography (GPC), using previous methodology,[56] in combination with a Agilent 1200 HPLC equipped with a PL aquagel-OH MIXED-H 8 μm column (300 × 7.5 mm, Polymer Laboratories Ltd.). The detection was achieved with a Knauer differential refractometer. The column temperature was maintained at 30 °C. The eluent was 0.02 N NaCl in 5 mM sodium phosphate buffer (pH 7.50), with a flow rate of 0.60 mL/min. The PL pullulan polysaccharide standards (peak average molecular weights 738, 12 200, 100 000, 1 600 000, Polymer Laboratories Ltd.) were used to obtain the calibration curve. The polysaccharide sample was dissolved with 0.02 N NaCl in 5 mM sodium phosphate buffer (pH 7.50) with a concentration of 2 mg/mL and filtered through a 0.45 μm filter membrane prior to analysis. The molecular weight distribution of the samples was calculated according to the calibration curve.

The samples were hydrolyzed with 1.0 M H_2SO_4 at 105 °C for 2.5 h for the monosaccharide composition analysis. Following hydrolysis, the samples were filtered and diluted to 50-fold, and then analyzed via high-performance anion-exchange chromatography (HPAEC) using a Dionex ICS3000 gradient pump, amperometric detector, AS50 autosampler, a Carbopac PA-20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex). Monosaccharides were separated in carbonate-free 18 mM NaOH under a N_2 atmosphere with a postcolumn addition of 0.3 M NaOH at a rate of 0.5 mL/min. The running time was 45 min, followed by 10 min elution with 0.2 M NaOH to wash the column. An additional 15 min elution was performed with 18 mM NaOH to re-equilibrate the column. The calibration was performed with standard solutions of L-rhamnose, L-arabinose, D-glucose,

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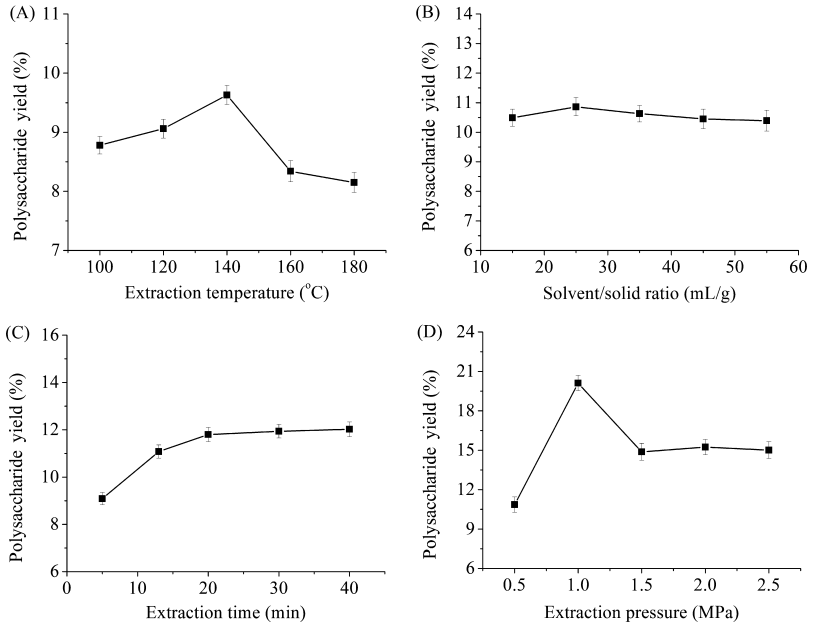

Figure 2. Influence of different extraction factors on polysaccharide yield ((A) extraction temperature; (B) solvent/solid ratio; (C) extraction time; and (D) extraction pressure). Values are means ± SD, n = 3.

D-xylose, D-mannose, D-galactose, glucuronic acid, and galacturonic acid.

2.8. Antioxidant Activity Assay. *2.8.1. Hydroxyl Radical Scavenging Ability.* The hydroxyl radical (•OH) scavenging ability of the purified polysaccharides was measured in accordance with previous methods.[56] Various concentrations (0−2 mg/mL) were incubated with 2 mmol/L $FeSO_4$ (1 mL), 0.1% H_2O_2 (1.0 mL), and 1 mL of 6 mmol/L salicylic acid (dissolved with alcohol) for 30 min at 37 °C. The hydroxyl radical was detected by monitoring the absorbance at 510 nm. The hydroxyl radical scavenging effect was calculated as follows: scavenging effect (%) = [1 − (Abs. of sample − Abs. of control)/Abs. of blank] × 100%. Salicylic acid was substituted with distilled water for the control. Ascorbic acid was used as the positive control. Lentinan extracted from mushroom was used as a comparison of other polysaccharide extracts.

2.8.2. ABTS Radical Scavenging Activity. The antioxidant activity was determined via 2,2′-azinobis[3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) radical cations as reported by Fan et al.,[57] with some modifications. The ABTS radical cation was produced by reacting 5 mL of 7 mmol/L ABTS diammonium salt with 0.088 mL of 140 mmol/L potassium persulfate. The mixture was placed in the dark for 24 h. When ready for use, the ABTS solution was diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm. Each sample (0.2 mL) with various concentrations (0.0625−2 mg/mL) was added to 3 mL of the diluted ABTS solution and mixed vigorously. The reaction mixture was allowed to stand at 25 °C for 60 min. The absorbance at 734 nm was quickly recorded. The ABTS scavenging effect was calculated as follows: effect (%) = [1 − (Abs. of sample − Abs. of control)/Abs. of blank] × 100%. Ascorbic acid was used as the positive control. Distilled water was substituted for the ABTS-diluted solution for the control. Distilled water was used instead of the sample for the blank. Lentinan extracted from mushroom was used as a comparison of other polysaccharide extracts.

3. RESULTS AND DISCUSSION

3.1. Single Factor Experimental Analysis. Effects of extraction temperature (°C), solvent/solid ratio (mL/g), extraction time (min), and extraction pressure (MPa) on polysaccharide yield (%) are shown in Figure 2. In Figure 2A, extractions are carried over varied intervals from 100 to 180 °C, while other extraction variables are set as follows: solvent/solid ratio at 15:1 (mL/g), extraction time at 12.5 min, and extraction pressure at 0.5 MPa. Results demonstrate that the maximum polysaccharide yield (9.63%) was obtained at 140 °C, from which point the yield began to decrease because of the partial degradation of polysaccharides. It is also observed in the SWE of other natural product.[58,59] The effect of the solvent/solid ratio on the polysaccharide yield was explored from 15:1 to 55:1 (mL/g) with an extraction temperature of 140 °C, extraction time at 12.5 min, and extraction pressure at 0.5 MPa. As shown in Figure 2B, the increased solvent/solid ratio had no obvious effect on polysaccharides yield. This indicates that solvent/solid ratio at 25:1 (mL/g) is sufficient to obtain high polysaccharides yield. Thus, the solvent/solid ratio was not a variable tested in future experiments. It is known that a short extraction time is beneficial to reduce the costs for industrial applications. As shown in Figure 2C, the maximum polysaccharide yield was obtained at 11.80% during a 12.5 min extraction interval, from which point the yield began to increase slightly. This might be explained by the research that active component yields will not continue to increase once equilibrium is reached.[60] Hence, 12.5 min was a suitable time for the extraction of polysaccharides from *D. nobile* Lindl. As detailed in Figure 2D (all other conditions fixed as described above), polysaccharide yields increased with increasing extraction pressure to 1.0 MPa, reaching their maximum yields (20.11%) when the temperature was 140 °C, solvent/solid ratio was 25:1 (mL/g), and time was 12.5 min. Yield of polysaccharides dropped when the pressure was more than 1.5 MPa, it may due to the destruction of the cellular structures of *D. nobile* Lindl. by heat and pressure and some dissolution of unfavorable ingredients.[61] Thus, extraction pressure of 1.0

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MPa was applicable. According to the results of a single-factor study, an extraction temperature of 120−160 °C, under an extraction time of 5−20 min, and an extraction pressure of 0.5−1.5 MPa were adopted for RSM experiments.

3.2. Optimization of SWE Conditions. *3.2.1. Box−Behnken Design.* The Box−Behnken statistical design with 3 factors (temperature, time, and pressure), over 3 levels and 15 runs, was selected for the optimization test. The observed responses (polysaccharide yield) are given in Table 1. The batch runs were performed with the BBD-designed experimental conditions, to visualize the effects that the independent factors had on the responses and on the results, for each experimental condition.[62] As the extraction temperature increased, the time and the pressure enhanced the polysaccharide yield. The results showed a wide range of differences between the polysaccharide yield. The maximum polysaccharide yield of 20.3% was achieved at 140 °C, for 12.5 min, and with 1.0 MPa (under conditions of run 15). The response given in Table 1 correlated to the three independent variables using a polynomial equation. The least squares regression was used to fit the data to eq 3. The best fit models in the coded factors were follows[63]

$$Y = 19.91 - 0.86A + 0.63B + 2.94C - 0.43AB + 0.70AC + 1.15BC - 0.91A^2 - 1.01B^2 - 6.49C^2 \quad (3)$$

where Y represented the multiple response, A was the temperature, B was the time, and C was the pressure.

The three-dimensional response surfaces were constructed from the BBD data. Figure 3 shows the interactions between the two variables by maintaining the third variable at level zero. The three-dimensional plots provided a visual interpretation of the interaction between two variables and facilitated the quantification of the optimum experimental conditions.[64] As seen in Figure 3a,c, the extraction temperature exhibited an important effect on the polysaccharide yield. The polysaccharide yield increased as the temperature increased to nearly 130 °C but as the temperature rose further, there was a gradual decrease in the polysaccharide yield because high temperature affected the polarity of subcritical water. This affected the solubility of the polysaccharides, which could result in the destruction of the polysaccharide's structure and lead to degradation.[42] Morales et al. also noticed the degradation of polysaccharides obtained from mushrooms.[65] This indicated that temperature plays an important role in the SWE extraction of polysaccharide. The effect that the extraction time had on the polysaccharide yield is presented in Figure 3a,b. The extraction yield increased when the time increased from 5 to 16.71 min, although increasing the time further only increased the extraction yield slightly. Extraction time did not demonstrate a significant effect on the polysaccharide yield. Figure 3b,c shows that the polysaccharide yield increased as the extraction pressure increased to the optimum level (1.12 MPa), and then decreased as the factor increased further. The higher pressure more easily destroyed the plant cell wall, which would increase the diffusion of the polysaccharides, although a much higher pressure caused the material to become too compact and the material in the middle of the vessel could not be in full contact with the water, thus decreasing the extraction yield.[42]

The optimum conditions obtained by the BBD were as follows: temperature = 129.83 °C, time = 16.71 min, and

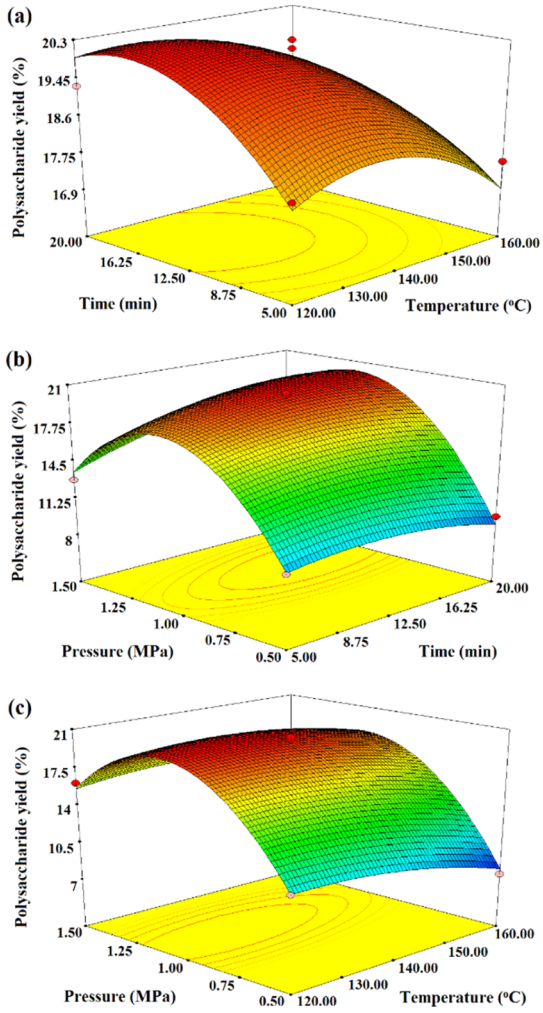

Figure 3. Response surfaces obtained from the BBD to the (a) temperature and time; (b) time and pressure; and (c) temperature and pressure.

pressure = 1.12 MPa. The maximum predicted that the polysaccharide yield was 20.67%, which corresponded fairly well to the real extraction yield (21.88%). This result indicated that the developed mathematical model was both accurate and adequate for predicting the polysaccharide extraction yield.

3.2.2. Analysis of Variance. The ANOVA test results are presented in Table 2. The model was determined to be adequate. The model F value of 46.83 demonstrated that the model was significant. There was a 0.03% chance that this large of a model F value could be due to noise. The values of "Prob > F" less than 0.0500 (0.0003) indicated that the model terms were significant. The model's determination coefficient R^2 was 0.9883, suggesting that 98.83% of the variability in the response was explained by the model. The lack of fit (2.89) was not significant because the P-value was greater than 0.0500 (0.2673).[66] The P-value was used as a tool to confirm the significance of each coefficient, which could indicate the pattern of the interaction between the variables.[64] The smaller the P-value was, the more significant the corresponding coefficient was. X_1, X_3, X_2X_3, and X_3^2 were significant ($P < 0.05$), while X_2, X_1X_2, X_1X_3, X_1^2, and X_2^2 were not significant ($P > 0.05$). The model obtained fit well with the BBD data.

3.3. Extraction with Different Methods. Figure 4 shows the extraction time-dependent yield data (a) and the

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Table 2. ANOVA of the Quadratic Model for the Polysaccharide Yield

source	sum of squares	degrees of freedom	mean square	F value	p-value Prob > F
model	243.13	9	27.01	46.83	0.0003
X_1	5.92	1	5.92	10.26	0.0239
X_2	3.16	1	3.16	5.48	0.0663
X_3	68.97	1	68.97	119.57	0.0001
X_1X_2	0.75	1	0.75	1.30	0.3064
X_1X_3	1.97	1	1.97	3.42	0.1236
X_2X_3	5.34	1	5.34	9.25	0.0287
X_1^2	3.07	1	3.07	5.33	0.0690
X_2^2	3.77	1	3.77	6.53	0.0509
X_3^2	155.52	1	155.52	269.60	<0.0001
residual	2.88	5	0.58		
lack of fit	2.34	3	0.78	2.89	0.2673
pure error	0.54	2	0.27		
total	246.01	14			

$\ln\left(\frac{Y_m}{Y_m - Y}\right) - T$ data (b) of the *D. nobile* Lindl. polysaccharide over the stirring extraction, the refluxing extraction, the ultrasound extraction, and the SWE methods. As shown in Figure 4a, the polysaccharide yields rose observably as the extraction time increased from 5 to 30 min, although the yields did not have a clear change at 50 min. This was most likely due to the attainment of dynamic equilibrium between the internal and external particles during the period between 50 and 190 min. The extraction time at their maximum polysaccharide yields were approximately the same (50 min), although the polysaccharide yields were different. The polysaccharide yields decreased in the following order: SWE > ultrasonic extraction > refluxing extraction > stirring extraction. The corresponding polysaccharide yield at 190 min was 23.42, 19.72, 17.54, and 15.93%. The results indicated that the polysaccharide did not reach sufficient extraction levels via the latter three methods. The plot for eq 2 is presented in Figure 4b, where Table 3 provides the experimental estimate for the k-value of the kinetic parameters, which was highest for SWE, followed by ultrasound extraction and refluxing extraction, and the stirring extraction had the lowest yield. The results confirmed that the SWE at a certain pressure and temperature could change the water density, thus improving the solvency of the water and gaining additional water-soluble organic compounds.[42] The SWE of the *D. nobile* Lindl. polysaccharide was highly effective. The R^2 value in Table 3 suggests that the model correlated well

Table 3. k and R^2 Values for Different Extraction Methods

extraction methods	stirring extraction	reflux extraction	ultrasonic extraction	SWE
k (1/min)	0.00938	0.01243	0.01498	0.01879
R^2	0.86688	0.89110	0.88137	0.89913

with all of the experimental data, indicating that the model was appropriate for the analysis of extraction processes on natural extracts.

3.4. Molecular Weight and Monosaccharide Composition of Polysaccharides. The average molecular weights of the *D. nobile* Lindl. polysaccharides obtained via different extraction methods were calculated at 117.09, 86.72, 103.46, and 85.72 kDa, and the chromatograms are shown in Figure S1 (see Supporting Information). The polysaccharides had no absorbance at 280 nm, suggesting that these polysaccharides did not contain protein. As seen in Table 4, the

Table 4. Molecular Weight and Monosaccharide Composition of Polysaccharide from *D. nobile* Lindl. with Different Extraction Methods

sample	stirring extraction	reflux extraction	ultrasonic extraction	SWE
$M_w \times 10^3$ (g/mol)	117.09	86.72	103.46	85.72
$M_n \times 10^3$ (g/mol)	0.815	0.48	0.97	1.42
arabinose (%)	1.03	1.83	0.97	1.05
galactose (%)	1.45	2.43	1.5	1.6
glucose (%)	54.28	46.98	54.84	48.63
mannose (%)	43.25	48.76	42.7	48.73

polysaccharides extracted via the SWE have the lowest M_w 85.72 × 10^3. This demonstrated that the polysaccharides from the SWE had the highest antioxidant activity. Zha et al. indicated that higher antioxidant activities of polysaccharides are found as the molecular weight decreases.[67] The polysaccharide that was extracted via SWE had the lowest M_w/M_n value (60.37), indicating that the polysaccharide had a relatively low index of polydispersity and a relatively narrow molecular weight distribution. The molecular weight changes could due to the hot compressed water extraction.

HPAEC analysis of the monosaccharide composition demonstrated that the polysaccharide that was extracted by the SWE was composed of L-arabinose, D-galactose, D-glucose, and D-mannose with a 1.05:1.6:48.63:48.73 ratio. Figure S2 (see Supporting Information) and Table 4 show that the

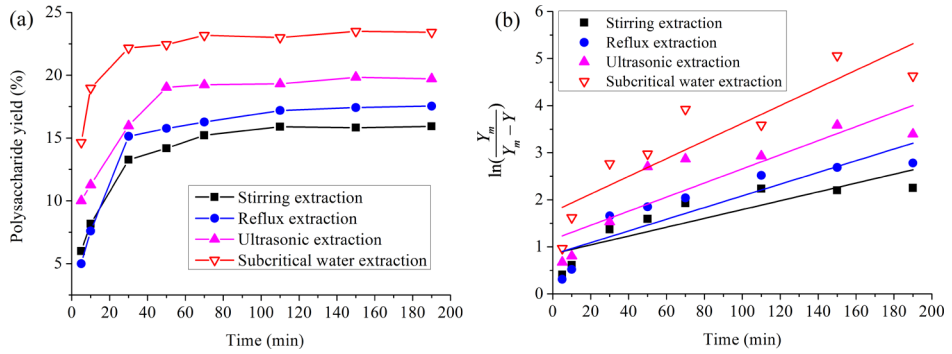

Figure 4. Time-dependent yield data (a) and $\ln\left(\frac{Y_m}{Y_m - Y}\right) - T$ data (b) of polysaccharide from *D. nobile* Lindl. at different conditions.

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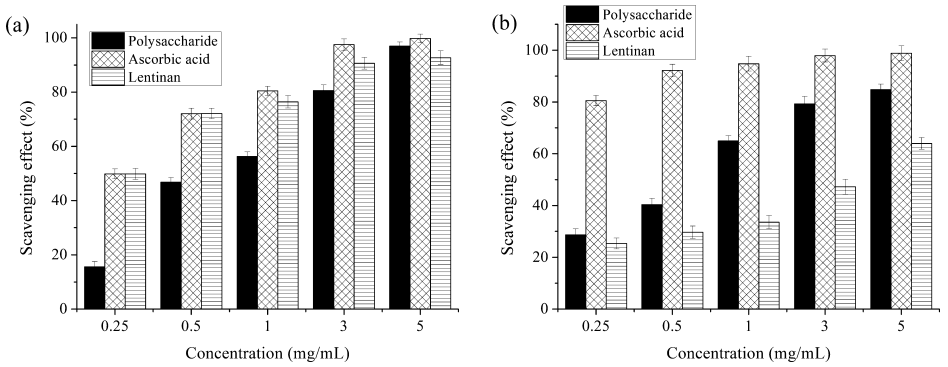

Figure 5. Scavenging effect of polysaccharide, ascorbic acid, and lentinan on hydroxyl radical (a) and ABTS radical (b). Values are means ± SD, $n = 3$.

monosaccharide composition of the *D. nobile* Lindl. polysaccharide obtained via various extraction methods were similar.

3.5. Antioxidant Activity Results. *3.5.1. Scavenging Effect on the Hydroxyl Radical.* The hydroxyl radical is the most active of the oxygen radicals, where it induces severe damage to the adjacent biomolecules.[68] As seen in Figure 5a, the hydroxyl radical scavenging activity of the polysaccharide is measured at various concentrations (0.25−5 mg/mL), with ascorbic acid as the positive control. These results suggested that the polysaccharide activity gradually rose as the concentrations increased. The ascorbic acid showed an excellent scavenging effect within 0.25−5 mg/mL. The polysaccharide and lentinan extracted from mushroom both exhibited high scavenging effects on the hydroxyl radical. The effect obtained 96.98 and 92.70% at 5 mg/mL, respectively, which was close to the ascorbic acid (99.74%). The results indicated that the *D. nobile* Lindl. polysaccharide displayed a strong scavenging power for the hydroxyl radical.

3.5.2. Scavenging Effect on ABTS Radical. The ABTS assay is often used when evaluating the total antioxidant power of single compounds and complex mixtures of natural products.[69] As Figure 5b shows, the polysaccharide, ascorbic acid, and lentinan extracted from mushroom demonstrated dose-dependent activities. The rate of polysaccharides, ascorbic acid, and lentinan scavenged ABTS radicals was 84.76, 98.85, and 63.96% at 5 mg/mL, respectively. The ABTS radical scavenging activity of the polysaccharide was inferior than the ascorbic acid but superior than lentinan. These results suggested that polysaccharide from different natural sources showed different radical scavenging abilities. In a word, the *D. nobile* Lindl. polysaccharide could be explored as novel potential antioxidants because of the remarkable antioxidant activity with a scavenging range between 28.66 and 84.76%.

4. CONCLUSIONS

SWE is a green process that was demonstrated to be an excellent method for *D. nobile* Lindl polysaccharide extraction. Single factor experiments and BBD of RSM were used to identify the optimal experimental conditions to be a time and pressure of 129.83 °C, 16.71 min, and 1.12 MPa. The maximal polysaccharide yield reached 21.88%. The polysaccharide was quantitatively determined via UV−vis spectrophotometry, where the results were compared with polysaccharides obtained by other methods (stirring extraction, refluxing extraction, ultrasound extraction, and SWE methods). The extraction processes were modeled and analyzed. The model had a good correlation between the experimental data and was determined to be fit for the comparison of the polysaccharide extraction. The polysaccharide extracted via SWE was composed of L-arabinose, D-galactose, D-glucose, and D-mannose at a 1.05:1.6:48.63:48.73 ratio. The average molecular weight was 85.72 kDa. The antioxidant activity assay demonstrated that the SWE had little effect on the medicinal properties of the polysaccharide. The rate of polysaccharide scavenging of hydroxyl radicals and the ABTS radical at 5.00 mg/mL were 96.98 and 84.76%. These results demonstrated that the SWE was an efficient and quick method for extracting polysaccharides and has potential as a useful technique for extracting other natural extracts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02550.

Additional images of GPC of polysaccharides and high-performance anion-exchange chromatographies of monosaccharide composition (PDF)

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Notes

The authors declare no competing financial interest.

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