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ORIGINAL ARTICLE

Optimization of Ultrasonic-Assisted Extraction of Bioactive Compounds from *Sargassum henslowianum* using Response Surface Methodology

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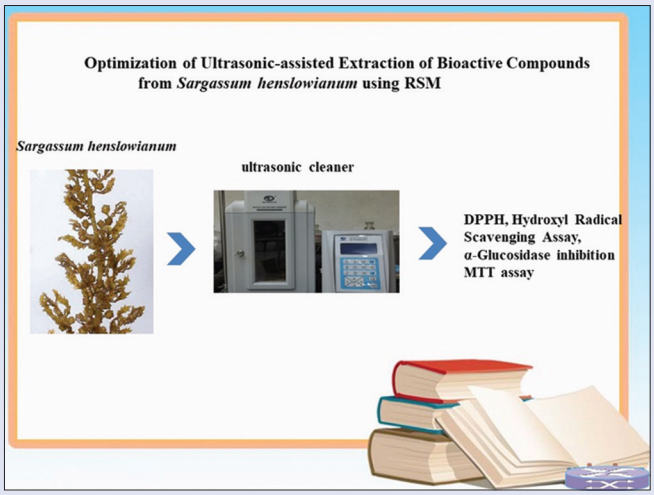
ABSTRACT

Background: *Sargassum henslowianum* has become an important source for the food industry as well as medicinal applications. In recent years, varieties of bioactive components in *S. henslowianum* and its activities have reported. However, the optimized extraction conditions of polysaccharides and polyphenols in *S. henslowianum* was unknown, and their activities need to be explored more. **Objective:** This study was to optimize the ultrasound-assisted extraction of polysaccharides and polyphenols from *S. henslowianum* and test the biological activity of the extract. **Materials and Methods:** Response surface methodology was used to optimize extraction conditions, and the antioxidant activity of the extracts was evaluated by radical scavenging assay, α-Glucosidase inhibition, and cytotoxicity on MCF-7. **Results:** The optimal conditions of extracting polysaccharides were shown as following: ultrasonic time for 40 min, ultrasonic power for 330 W, solid-to-liquid ratio for 1:36 g/mL, the extraction yield reached 12.63% under above parameters. The optimum conditions of ultrasonic-assisted extraction of total polyphenols were as following: ultrasonic time for 102 min, ultrasonic power for 377 W, alcohol concentration for 62%, under these conditions, the extraction yield reached 11.45%. Besides, the extracts of polyphenols possessed stronger activity. **Conclusion:** This study provides the scientific guidance for further exploitation and utilization of *S. henslowianum*.

Key words: Bioactivity, response surface methodology, *Sargassum henslowianum*, ultrasonic extraction

SUMMARY

- Response surface methodology was used to optimize extraction conditions, and the antioxidant activity of the extracts was evaluated by radical scavenging assay, α-Glucosidase inhibition, and cytotoxicity on MCF-7. The optimal conditions of extracting polysaccharides were shown as following: ultrasonic time for 40 min, ultrasonic power for 330 W, a solid-to-liquid ratio for 1:36 g/mL, the extraction yield reached 12.63% under above parameters. The optimum conditions of ultrasonic-assisted extraction of total polyphenols were as following: ultrasonic time for 102 min, ultrasonic power for 377 W, alcohol concentration for 62%, and the extraction yield reached 11.45% under these conditions. Besides, the extracts of polyphenols possessed stronger activity.



Abbreviations used: RSM: Response Surface Methodology; BBD: Box-Behnken Design; ANOVA: Analysis of variance

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INTRODUCTION

Sargassum henslowianum, which belongs to genus of brown seaweed, fucales, sargassaceae family, grows on low-tide zone rocks, and extensively distributes in Hong Kong and Guangdong province. However, the utilization of *S. henslowianum* is restricted, and only a small part play a role as the raw materials of feed, algae and pharmaceutical industry.[1] In recent years, a wealth of bioactive components such as meroterpenoids, phlorotannins, polysaccharides, dietary fiber, and phytosterols have been identified in *S. henslowianum*[2] and the pharmacological properties such as internal heat, infections, laryngitis, anticancer, antibacterial, antifungal, antiviral, and anti-inflammatory have been recognized.[3-6] Consequently, extensive attention has been paid to its health care value and medicinal value. The polysaccharides in *S. henslowianum* mainly consist of fucoidan, alginate, and laminaran, which possess a wide range of biological activity, such as anticancer, anti-inflammatory, anticoagulant, and hypolipidemic.[7,8] The polyphenols of fucophlorethols, fuhalols, and phlorethols were proved in *S. henslowianum,* and the anticoagulant and antioxidant activities have been reported.[9] To further develop the

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marine resources and expand application range of *S. henslowianum,* it is necessary to optimize extraction conditions of bioactive components.

Maceration and percolation are considered as traditional methods to extract biological active compounds from plant materials. To obtain higher quality biological active compounds efficiently, different methods such as ultrasonic-assisted extraction have been reported.[10] Owing to the cavitation, mechanical and thermal effects of ultrasound, the release, diffusion, and dissolution of the active substances in the cells are accelerated.[11] Ultrasonic-assisted extraction possesses the advantage of high efficiency, time-saving, and environmental kindness compared to classical heating extraction and Soxhlet extraction.[12]

The objective of this study was to investigate the optimum ultrasonic-assisted extraction process of polysaccharides and polyphenols and study the biological activity of the extracts from many aspects. Response Surface Methodology (RSM) was applied to analyze the relationship between factors and response value. Moreover, diphenyl picryl hydrazinyl (DPPH), hydroxyl radical scavenging, α-Glucosidase inhibition, and cytotoxicity on MCF-7 assay were used to investigate the activity of polysaccharides and polyphenols from *S. henslowianum*.

MATERIALS AND METHODS

Materials

S. henslowianum were purchased from Zhanjiang (Guangdong, China). Glucose and gallic acid standards were from Mann Stewart Biological Technology Co. Ltd. (Chengdu, China). α-Glucosidase was purchased from Sigma Chemical Co., (America). Other reagents were analytical reagent and from Fu Chen Chemical Reagent Factory (Tianjin, China). This article does not contain any studies with human or animal subjects.

Preparation of calibration curve of glucose

The calibration curve of glucose was determined by the phenol-sulfuric acid method.[13] Briefly, different concentrations of glucose solution (0.02, 0.04, 0.06, 0.08, 0.1, 0.12 mg/mL) were prepared, and 1.0 mL was taken into test tube. Then, 1.0 mL 5% phenol solution was added slowly under the conditions of ice water bath, shaken, 5 mL sulfuric acid was added immediately and the mixture was shaken for 5 min. The resulting solution in boiling water bathed for 10 min and in water bath for 20 min. The absorbance was measured at 490 nm using UV-Vis spectrophotometer (752, Shanghai, China). The standard curve was prepared with the concentration of glucose as the abscissa and the absorbance value as the ordinate.

Preparation of calibration curve of gallic acid

The calibration curve of gallic acid was determined by Folin–Ciocalteu reagent.[14] Briefly, different concentrations of gallic acid solution (0.02, 0.04, 0.06, 0.08, 0.1, 0.12 mg/mL) were prepared. 0.5 mL Folin was added and reacted for 5 min, and then added 1.5 mL 20% sodium carbonate solution. The mixture was incubated for 30 min at room temperature. The absorbance was measured at 763 nm by UV-Vis spectrophotometer (752, Shanghai, China). The standard curve was prepared with the concentration of gallic acid as the abscissa and the absorbance value as the ordinate.

Extraction of polysaccharides and polyphenols

The *S. henslowianum* were ground into powder by a pulverizer (DFY-600, Wenling, China) and passed through 40 mesh sieve. 1.0 g powder was used to extract target compounds with ultrasonic cleaner (KQ-400DB, Dongguan, China) under different conditions. After the extraction process, the supernatant was collected by filtration. The content of polysaccharides and polyphenols in the extract were determined by the method shown in the above section. The extraction rate was calculated by the following formula:

$$Y\ (\%) = \frac{C \times V \times 10^{-3}}{W} \quad (1)$$

Where C (mg/mL) is the concentration of Sargassum polysaccharide or polyphenols, V (mL) is the volume of extraction, W (g) is the quality of Sargassum powder.

Single-factor test

Single-factor test was used to provide a guide for the experimental design of RSM. In this test, one factor was changed while the other factors were kept constant, so the influence of each factor on extraction rate was revealed. The extraction factors of polysaccharides included extraction time (X_1), ultrasonic power (X_2) and solid-to-liquid ratio (X_3); and of polyphenols included extraction time (X_1), ultrasonic power (X_2), and alcohol concentration (X_3).

Experimental design of response surface methodology

Box-Behnken Design with three-level-three-factor was employed to optimize the extraction yield of polysaccharides and polyphenols.[15] The independent variables were coded at three levels including-1, 0 and 1, and the extraction yield was deemed to the response. The experiment was designed using Design-Expert software version 8.0.5. The variables and their levels were shown in Table 1 for polysaccharides and Table 2 for polyphenols. Each trial was performed in triplicate. A second-order polynomial was obtained by the experimental data,[16] and the form was shown as follows:

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

Where Y is extraction yield, β_0, β_i, β_{ii}, and β_{ij} represent the interception, linear coefficient, quadratic coefficient, and interaction coefficient, respectively.

Antioxidant activity assay

Diphenyl picryl hydrazinyl radical scavenging assay

The extracts of seaweed polysaccharides and polyphenols were obtained according to the optimal conditions, respectively. The DPPH free radical scavenging activity of polysaccharides and polyphenols extracted from *S. henslowianum* was analyzed using the method described by this[17] with minor modification. Briefly, 5 mL extracts at the different concentration of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL was mixed with 5 mL of 0.04 mg/mL DPPH-ethanol solution respectively, and then reacted at room temperature for 30 min. The absorbance was measured at 517 nm.

$$\text{DPPH radical scavenging activity}\ (\%) = \left[1 - \frac{A_i - A_j}{A_0}\right] \times 100 \quad (3)$$

Where A_i is the absorbance of the sample, A_j is the absorbance of the mixture of ethanol and sample, A_0 is the absorbance of the control solution.

Hydroxyl radical scavenging assay

The method proposed by this[18] was used to investigate the scavenging effect of seaweed polysaccharides and polyphenols on hydroxyl radical with a minor modification. Briefly, the reaction solution contained 2 mL Fe^{2+} (1.5 mmol/L), 1 mL salicylic acid (3 mmol/L), 2 mL H_2O_2 (0.3%), and different concentrations of seaweed polysaccharides or polyphenols.

Table 1: Factors and levels for polysaccharides and central composite design with the independent variables

Run	X_1 (extraction time, min)	X_2 (ultrasonic power, W)	X_3 (solid-to-liquid ratio, g/mL)	Y (extraction yield, %)
1	0 (40)	0 (320)	0 (1:35)	12.78
2	1 (50)	0 (320)	0 (1:35)	11.36
3	1 (50)	1 (360)	1 (1:40)	11.03
4	0 (40)	1 (360)	0 (1:35)	12.14
5	−1 (30)	1 (360)	−1 (1:30)	9.43
6	0 (40)	0 (320)	0 (1:35)	12.78
7	−1 (30)	0 (320)	0 (1:35)	11.82
8	−1 (30)	1 (360)	1 (1:40)	10.9
9	0 (40)	0 (320)	0 (1:35)	12.8
10	1 (50)	−1 (280)	−1 (1:30)	9.39
11	1 (50)	1 (360)	−1 (1:30)	9.3
12	0 (40)	0 (320)	1 (1:40)	12.45
13	0 (40)	0 (320)	0 (1:35)	12.8
14	0 (40)	−1 (280)	0 (1:35)	12.17
15	0 (40)	0 (320)	−1 (1:30)	11.02
16	1 (50)	−1 (280)	1 (1:40)	9.93
17	0 (40)	0 (320)	0 (1:35)	12.8
18	0 (40)	0 (320)	0 (1:35)	12.81
19	−1 (30)	−1 (280)	1 (1:40)	9.38
20	−1 (30)	1 (360)	−1 (1:30)	9.17

Table 2: Factors and levels for polyphenols and central composite design with the independent variables

Run	X_1 (extraction time, min)	X_2 (ultrasonic power, W)	X_3 (alcohol concentration, %)	Y (extraction yield, %)
1	0 (100)	0 (360)	0 (60)	11.66
2	1 (120)	0 (360)	0 (60)	10.74
3	1 (120)	1 (400)	1 (70)	10.01
4	0 (100)	1 (400)	0 (60)	11.04
5	−1 (80)	1 (400)	−1 (50)	8.37
6	0 (100)	0 (360)	0 (60)	11.59
7	−1 (80)	0 (360)	0 (60)	10.72
8	−1 (80)	1 (400)	1 (70)	10.02
9	0 (100)	0 (360)	0 (60)	11.75
10	1 (120)	−1 (320)	−1 (50)	8.42
11	1 (120)	1 (400)	−1 (50)	8.22
12	0 (100)	0 (360)	1 (70)	11.34
13	0 (100)	0 (360)	0 (60)	11.76
14	0 (100)	−1 (320)	0 (60)	11.09
15	0 (100)	0 (360)	−1 (50)	10
16	1 (120)	−1 (320)	1 (70)	8.85
17	0 (100)	0 (360)	0 (60)	11.59
18	0 (100)	0 (360)	0 (60)	11.74
19	−1 (80)	−1 (320)	1 (70)	8.5
20	−1 (80)	1 (400)	−1 (50)	8.06

The mixtures were incubated for 30 min at 37°C, and the absorbance was measured at 510 nm.

$$\text{Hydroxyl radical scavenging activity}\,(\%) = \left(1 - \frac{A_i - A_{i0}}{A_0}\right) \times 100 \quad (4)$$

Where A_i is the absorbance of the sample solution, A_0 is the absorbance of control solution (water instead of the sample), A_{i0} is the absorbance value of the sample under identical condition as A_i with water instead of H_2O_2.

α-glucosidase inhibition

The α-Glucosidase inhibition activity of the extracts from *S. henslowianum* was measured by this.[19] Briefly, 100 μL phosphate buffer (PH 6.8), 20 μL α-Glucosidase and 10 μL sample solution in different concentration were added into 96-well plate in sequence, then the mixture was stored in 37°C for 15 min and acarbose as a positive control. Followed by adding 20 μL PNPG and incubating at 37°C for 20 min. The OD value was measured at 405 nm using microplate reader (ST-360, Shanghai, China).

3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay

MCF-7 cells (5×10^4 cells/well) were treated with *S. henslowianum* extracts whose concentration were in the range of 50–1600 μg/mL, and then processed cells were incubated for 24 h. Cell viability was measured by ELISA plate reader using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay.[20] Each point set three replicated trials, and the results were averaged.

RESULTS AND DISCUSSION

Effect of extraction time on extraction yield

To investigate the effect of extraction time on extraction yield of polysaccharides, extraction time ranged from 10 to 60 min while the ultrasonic temperature, solid-to-liquid ratio, and ultrasonic power

were fixed at 40°C, 1:25, and 280 W, respectively. With the increasing of ultrasonic time, the extraction yield of polysaccharides increased and reached a plateau at 40 min and then decreased slightly, as shown in Figure 1a. While for polyphenols, extraction time varied from 20 to 100 min, and the ultrasonic temperature, alcohol concentration, and ultrasonic power were 60°C, 60%, and 400 W, respectively. Figure 2a exhibits that with the increasing of extraction time from 20 to 100 min, the content of polyphenols increased, and then tended to descend.

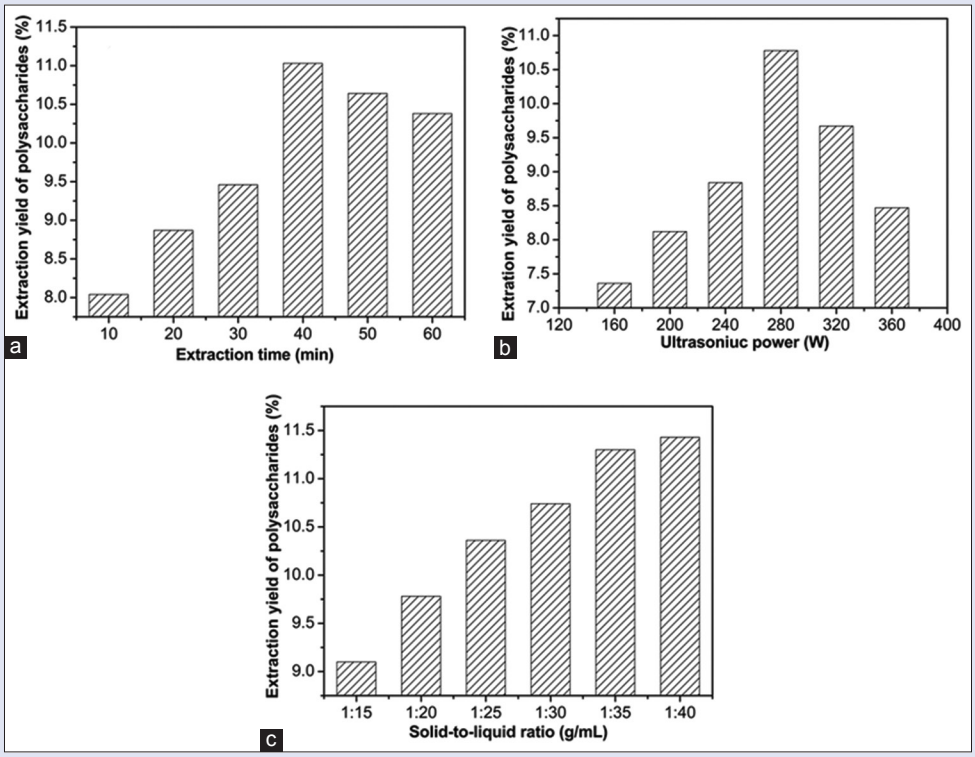

Figure 1: Effects of different parameters on the extraction yield of polysaccharides ([a]: Extraction time, [b]: Ultrasonic power, [c]: Solid-to-liquid ratio)

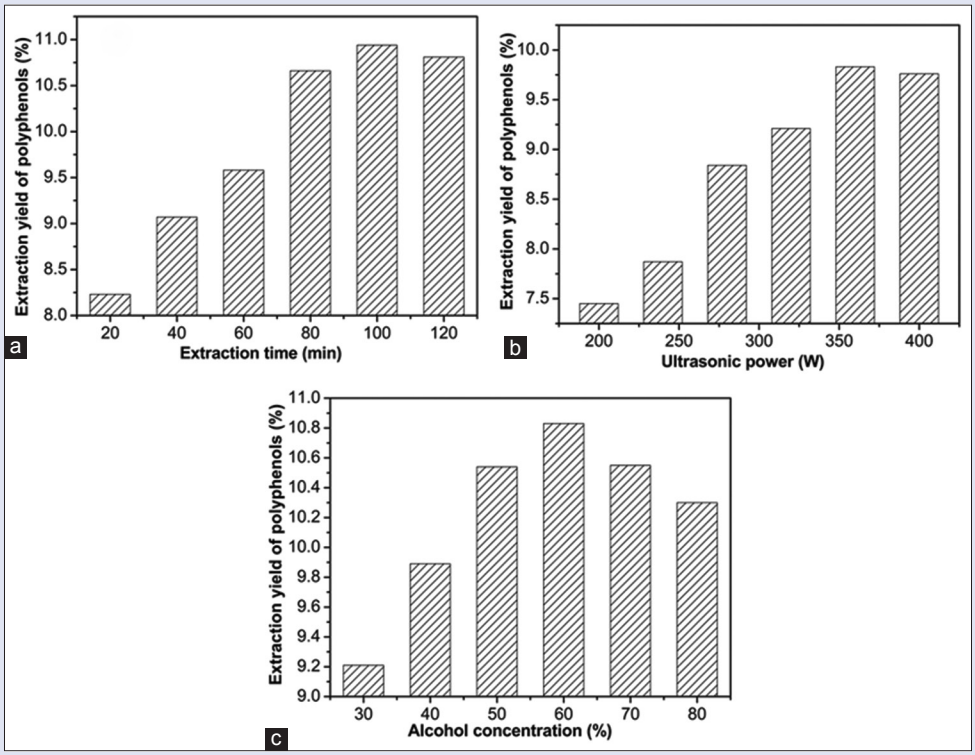

Figure 2: Effects of different parameters on the extraction yield of polyphenols ([a]: Extraction time, [b]: Ultrasonic power, [c]: Alcohol concentration)

Initially, the content of the target compound in the extraction solvent was in low level and the extension of time is beneficial to the dissolution of the target compound. However, the structure of polysaccharides and polyphenols were destroyed as time prolonged. Considering the economic benefit, the extraction time should not be chosen for a long time but a moderate time.[21,22]

Effect of ultrasonic power on extraction yield

Ultrasonic power changed in the range of 160 W and 360 W while the ultrasonic time, temperature, and solid-to-liquid ratio were set as 30 min, 40°C, and 1:25 to extract polysaccharides. For polyphenols, ultrasonic power changed in the range of 200 and 400 W and the extraction time, temperature and alcohol concentration were set as 60 min, 60°C, and 60%. According to the results of Figures 1b and 2b, with the increasing of ultrasonic power, the extraction rate of polysaccharides and polyphenols rise rapidly and then dropped. Furthermore, the extraction rate reached maximum value when the ultrasonic power was 280 W for polysaccharides and 320 W for polyphenols. The destructive effect of ultrasound on the cell wall was beneficial to the dissolution of compound, however, once the power was too high, the damage of ultrasound for the target compound was enhanced obviously. Hence, it is necessary to investigate the optimal ultrasonic power.[23-25]

Effect of solid-to-liquid ratio on extraction yield of polysaccharides

The value of solid-to-liquid ratio was chosen including 1:15, 1:20, 1:30, 1:35, and 1:40. Others were fixed as the followings: ultrasonic time 30 min, temperature 40°C, and ultrasonic power 280 W. Figure 1c shows that with the increasing of extraction solvent, the extraction rate of polysaccharides increased rapidly and then tended to be stable. With a small volume of extraction solvent, the polysaccharide dissolved in the solution to reach saturation easily, which leads to low extraction rate. The increasing of solvent was beneficial to the dissolution and diffusion of polysaccharides and caused a significant increase in the extraction yield.[26-28]

Effect of alcohol concentration on polyphenols yield

The value of alcohol concentration was chosen including 30%, 40%, 50%, 60%, 70%, and 80%. Other parameters were fixed as the followings: ultrasonic time 60 min, temperature 60°C, and ultrasonic power 400 W. Figure 2c indicates that under the condition of 60% ethanol, the extraction rate reached the highest value, the main reason was that the polarity between 60% ethanol and polyphenols was similar, which was beneficial to the dissolution of polyphenols.[29] Therefore, it is significant to choose the appropriate solvent for higher extraction yield.

Response surface analysis test

In the single factor test, extraction variables of polysaccharides (extraction time, ultrasonic power, and solid-to-liquid ratio) and polyphenols (extraction time, ultrasonic power, and alcohol concentration) were investigated, respectively. Under these results, the Center Combination Design (CCD) was used to optimize the ultrasonic extraction conditions, which included factorial point and zero point and zero-point experiments were repeated six times to estimate the experimental error.

Optimization of extraction conditions of polysaccharides

Tables 1 and 2 represent the result of CCD experiments of polysaccharides and polyphenols respectively, and the second-order polynomial obtained by the experimental data was expressed by the following equation as an indication of coded factors.

$$Y_{polysaccharide} = 12.81 + 0.031X_1 + 0.28X_2 + 0.054X_3 - 0.096X_1X_2 + 0.074X_1X_3 + 0.31X_2X_3 - 1.23X_1^2 - 0.67X_2^2 - 1.09X_3^2 \quad (5)$$

$$Y_{polyphenol} = 11.72 + 0.057X_1 + 0.27X_2 + 0.56X_3 - 0.11X_1X_2 + 0.016X_1X_3 + 0.32X_2X_3 - 1.06X_1^2 - 0.72X_2^2 - 1.12X_3^2 \quad (6)$$

Where $Y_{polysaccharides}$ is the extraction yield of polysaccharides, X_1, X_2, X_3 represent the independent variables of extraction time (min), ultrasonic power (W), and solid-to-liquid ratio (g/mL) for equation (5); for equation (6), where $Y_{polyphenols}$ is the extraction yield of polyphenols, X_1, X_2, X_3 represent the independent variables of extraction time (min), ultrasonic power (W), and alcohol concentration (%).

The validity of the model was tested using analysis of variance (ANOVA) and the result of ANOVA for quadratic polynomial model of extraction of polysaccharides and polyphenols were shown in Tables 3 and 4, respectively.

Analysis of response surface

Results of ANOVA for the fitted quadratic polynomial model of extraction of polysaccharides were shown in Table 3. The value of R^2 was 98.78%, indicating that 98.87% of the variables (extraction time, ultrasonic power, and solid-to-liquid ratio) could be explained using the model obtained

Table 3: Results of analysis of variance for the fitted quadratic polynomial model of extraction of polysaccharides

Source	Degree of freedom	Sum of square	Mean square	*F*	*P*
Model	9	37.56	4.17	89.93	<0.0001
X_1	1	9.61E-03	9.61E-03	0.21	0.6588
X_2	1	0.76	0.76	16.41	0.0023
X_3	1	2.89	2.89	62.37	<0.0001
X_1X_2	1	0.074	0.074	1.6	0.235
X_1X_3	1	0.044	0.044	0.94	0.3557
X_2X_3	1	0.75	0.75	16.17	0.0024
X_1^2	1	4.17	4.17	89.92	<0.0001
X_2^2	1	1.22	1.22	26.35	0.0004
X_3^2	1	3.25	3.25	70	<0.0001
Residual	10	0.46	0.046		
Lack of fit	5	0.46	0.093	617.75	<0.0001
Pure error	5	7.50E-04	1.50E-04		
Cor.total	19	38.02			

R^2=0.9878; R^2_{Adj}=0.9768; R^2_{Pred}=0.9054; Adeq precisior=24.242; CV (%)=1.9

Table 4: Results of analysis of variance for the fitted quadratic polynomial model of extraction of polyphenols

Source	Degree of freedom	Sum of square	Mean square	*F*	*P*
Model	9	35.94	3.99	121.6	<0.0001
X_1	1	0.032	0.032	0.99	0.3433
X_2	1	0.75	0.75	22.86	0.0007
X_3	1	3.19	3.19	97.22	<0.0001
X_1X_2	1	0.095	0.095	2.89	0.1205
X_1X_3	1	2.112E-03	2.112E-03	0.064	0.8049
X_2X_3	1	0.83	0.83	25.14	0.0005
X_1^2	1	3.08	3.08	93.86	<0.0001
X_2^2	1	1.44	1.44	43.86	<0.0001
X_3^2	1	3.44	3.44	104.8	<0.0001
Residual	10	0.33	0.33		
Lack of fit	5	0.30	0.060	9.94	0.0124
Pure error	5	0.030	6.000E-03		
Cor.total	19	36.26			

R^2=0.9909; R^2_{Adj}=0.9828; R^2_{Pred}=0.9377; Adeq precisior=27.949; CV (%)=1.76

by the experiment. The R^2_{Pred} of 90.54% was in reasonable agreement with the R^2_{Adj} of 97.68%. The value of R^2_{Adeq} was 24.242, which was >4, indicated an adequate signal. Above data certified that this model could be used to navigate the design space. Quadratic regression model *F*-value of 89.93 implied the model was significant. Values of "Prob > *F*" <0.05 indicated the term was significant. In this case, independent variables (X_2, X_3), interaction coefficient (X_2X_3), and quadratic terms (X_1^2, X_2^2, X_3^2) were significant. Results of ANOVA for the fitted quadratic polynomial model of extraction of polyphenols were shown in Table 4, the value of R^2 was 99.09%, which meant that 99.09% of the variables (extraction time, ultrasonic power, and alcohol concentration) could be explained using the model obtained by the experiment. The R^2_{Pred} of 93.77% was in reasonable agreement with the R^2_{Adj} of 98.28%. The value of R^2_{Adeq} was 27.949, which was >4, indicated an adequate signal. Above data certified that this model can be used to navigate the design space. Quadratic regression model *F*-value of 121.60 implied the model was significant. The values of "Prob > *F*" <0.05 indicated the term was significant. In this case, independent variables (X_2, X_3), interaction coefficient (X_2X_3), and quadratic terms (X_1^2, X_2^2, X_3^2) were significant.

The three-dimensional profiles of polysaccharides and polyphenols were shown in Figures 3 and 4, respectively, which illustrated the relationship between dependent variables and the independent. In addition, different shapes represent different interactions (the greater the bending amplitude of the response surface plots, the variables more significant). Figure 3c was the steepest response surface plot, which meant that the variable of solid-to-liquid ratio was more significant than extraction time and ultrasonic power and this result was shown in Table 3. Figure 4c was the steepest response surface plot, which meant that the variable of alcohol concentration was more significant than extraction time and ultrasonic power. This result was in agreement with the result in Table 4.

Optimization of the extraction condition and validation

Through the model was obtained by experiment, the optimum conditions of extraction polysaccharides from *S. henslowianum* were as follows: extraction time for 40.1 min, ultrasonic power for 330.88 W, solid-to-liquid ratio for 1:36.43, and in consideration of the actual operation, the best extraction process was modified to extraction time 40 min, ultrasonic power 330 W, solid-to-liquid ratio for 1:36. Under the above condition, the extraction yield reached 12.63% (n = 3), which approached predicted value of 12.92%.

For polyphenols, the optimum conditions were shown as following: extraction time for 102.33 min, ultrasonic power for 377.12 W, alcohol concentration for 62.75%, and considering the actual operation, the extraction condition was modified to extraction time 102 min, ultrasonic power 377 W, alcohol concentration 63%. Under these conditions, the extraction yield reached 11.45% (n = 3), which was close to the predicted value of 11.72%.

Antioxidant activity analysis

DPPH and hydroxyl radical scavenging assay were applied to investigate the antioxidant activity of polysaccharides and polyphenols extracted from *S. henslowianum*, and the results were shown in Figure 5a and b. The DPPH and hydroxyl radical scavenging ability increased with the increase in concentrations of polysaccharides and polyphenols. Polyphenols possessed stronger scavenging activity on both DPPH and hydroxyl radical by comparing the results. Furthermore, both extracts showed good scavenging activity on DPPH and hydroxyl radical in the same concentration.

α-glucosidase inhibition

In this experiment, the inhibition rate of *S. henslowianum* extracts to α-Glucosidase was measured by enzyme dynamic experiment, and the value of IC_{50} was used to compare the ability of two different extracts inhibiting α-Glucosidase. According to the OD value, the IC_{50} value of polysaccharides, polyphenols, and acarbose was 1844.6, 832.7, and 1256.2 μg/mL. It was easy to get the conclusion that *S. henslowianum* polyphenols possessed the stronger inhibitory activity of α-Glucosidase than polysaccharides and positive control, which meant that polyphenols had a potential hypoglycemic value.

3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay

Figure 5c summarizes the result of MTT assay, which indicated that increasing dose inhibited proliferation of cells more. By calculation,

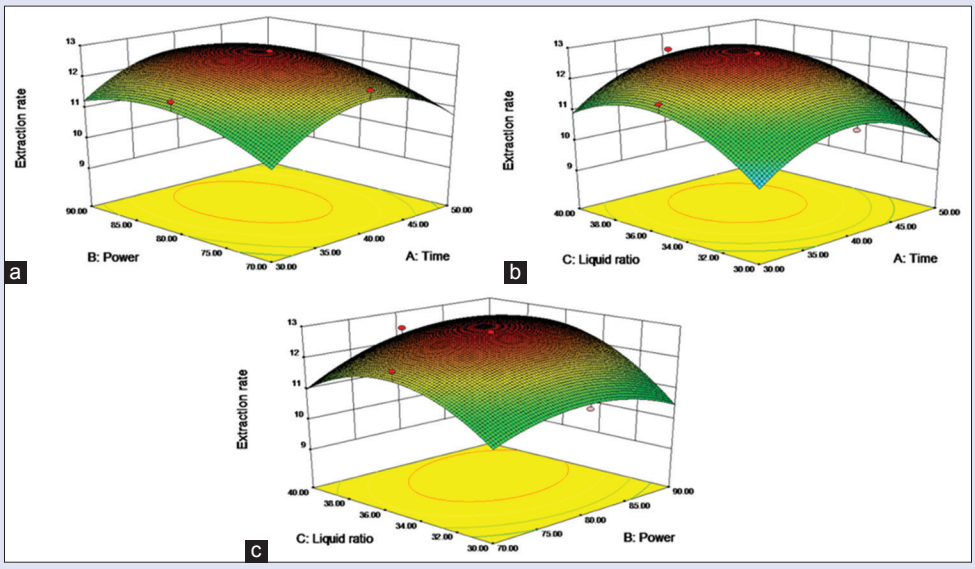

Figure 3: Response surface plots showing the effect of different variables on the extraction rate of polysaccharides ((a) Extraction time and ultrasonic power, (b) Extraction time and solid-to-liquid ratio, (c) Ultrasonic power and solid-to-liquid ratio)

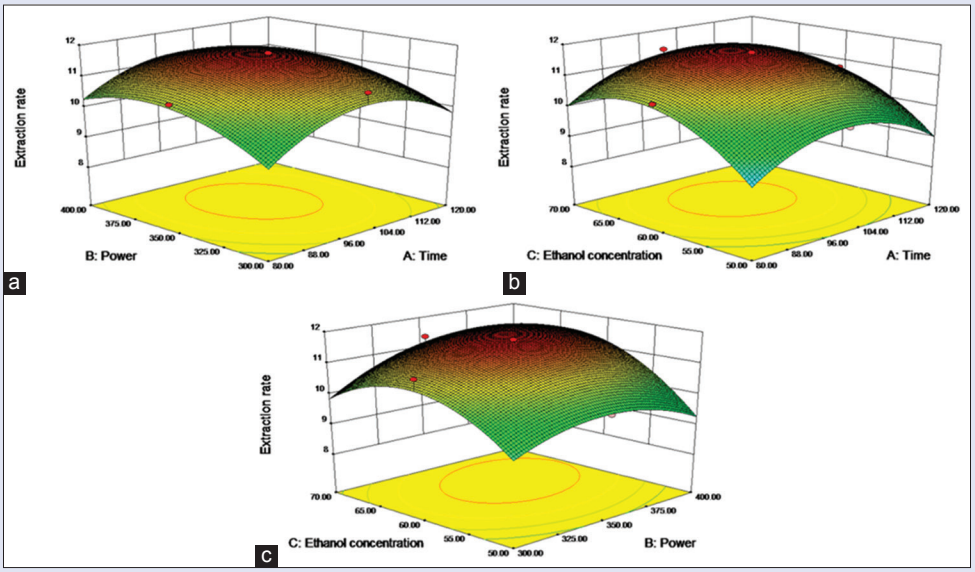

Figure 4: Response surface plots showing the effect of different variables on the extraction rate of polyphenols ((a) Extraction time and ultrasonic power, (b) Extraction time and alcohol concentration, (c): Ultrasonic power and alcohol concentration)

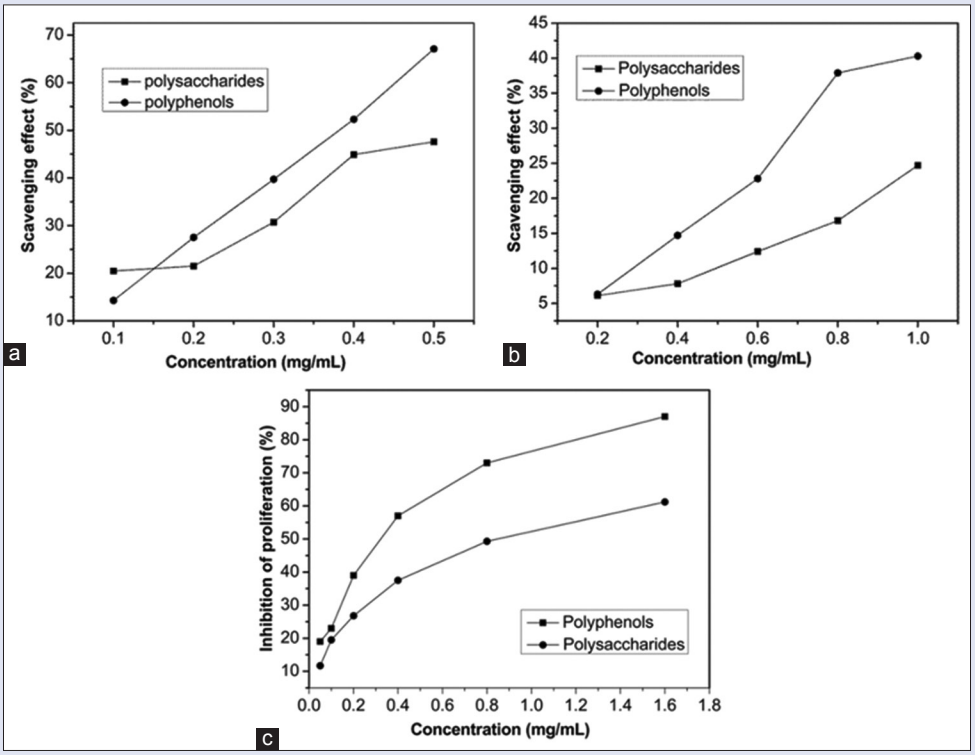

Figure 5: Activities of polysaccharides and polyphenols. (a) Diphenyl picryl hydrazinyl; (b) hydroxyl radical; (c) Inhibitory activity of polysaccharides and polyphenols on MCF-7

the IC_{50} of polysaccharides, polyphenols was 776 and 288 μg/mL, this phenomenon proved polyphenols possessed a stronger inhibitory effect on MCF-7.

CONCLUSION

RSM was applied to optimize ultrasonic-assisted extraction conditions of *S. henslowianum* polysaccharides and polyphenols and both models obtained through this study can be used to predict the experimental value. The optimal conditions for extracting polysaccharides were as following: ultrasonic time for 40 min, ultrasonic power for 330 W, solid-to-liquid ratio for 1:36, and the extraction yield reached 12.63% under these parameters. The optimum conditions for ultrasonic-assisted extraction of total polyphenols were as following: ultrasonic time for 102 min, ultrasonic power for 377 W, and alcohol concentration for 62%. Under these conditions, the extraction yield reached 11.45%. The antioxidant assay, α-Glucosidase inhibition,

and MTT assay indicated that polyphenols possessed stronger activity than polysaccharides. This study provides scientific guidance for further exploitation and utilization of *S. henslowianum*.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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