Polysaccharides from *Bletilla striata*: Extraction, Optimization and Their Antioxidant Activities *in vitro*

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Abstract In this paper, the extraction conditions of water-soluble polysaccharide from *Bletilla striata* were optimized by response surface methodology. With the dried *Bletilla striata* as raw materials, phenol-sulfuric acid method as the method of polysaccharide extraction rate, polysaccharide extraction rate as an indicator, the optimum process conditions for polysaccharide extraction from *Bletilla striata* were obtained including liquid-solid ratio of 15 mg/mL, extraction temperature 90 °C, extraction time of 90 h, sample immersion time 90 min, and extraction 2 times, achieving the corresponding polysaccharide yield of 48.38%. The antioxidant assays *in vitro* revealed that the polysaccharides from *Bletilla striata* exhibited a slight scavenging activity for 1,1-diphenyl-2-picrylhydrazyl radicals and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cations.

Keywords *Bletilla striata*, polysaccharides, extraction, antioxidant activities

Introduction

Bletilla striata is a health food, which is the dried tuber of *Bletilla Striata* (Thunb.) Reichb.f and has a long medicinal history.^[1] It is mainly distributed in Guizhou, Sichuan, Hunan, Jiangxi and Guangxi provinces in China. *Bletilla striata* is also a traditional Chinese medicine, which is widely used by Chinese. It has several effects of astringency and hemostasis, clearing heat and promoting diuresis, detumescence and promoting granulation. Its pharmacological activities mainly include anti-bacterial, hemostasis, anti-tumor, anti-ulcer, anti-fibrosis, anti-oxidation, promoting wound healing, etc. Thus, *Bletilla striata* is widely used in clinic to treat hemoptysis, hematemesis, trauma bleeding, sores and swelling poison, skin chapping, anorectal diseases, gynecological fibroids, tumor embolism and prostate surgery, and so on.^[2-8] *Bletilla striata* contains several phytochemical compounds such as phenolic compounds, dihydrophenanthrene, phenanthrene, bibenzyl and polysaccharides.^[9] Polysaccharides are a class of macromolecular compounds that contain more than ten monosaccharides, and they are polymerized by glycosidic bonds. Polysaccharides commonly exist in animals, plants, microorganisms and algae. Some studies have confirmed polysaccharides possess various bioactivities including antioxidant, antitumor and immunoregulatory activities.^[10-13]

This present work was aimed to optimize the extraction conditions for maximum yield of *Bletilla striata* polysaccharide (BSP) using response surface methodology and evaluate their *in vitro* antioxidant activity.

Materials and Methods

Materials and chemicals

Bletilla striata was purchased from Liangbao Medicinal Materials Company in Puer city (Yunnan, China) in 2017. The

dry *Bletilla striata* was crushed with a pulverizer and the 60—80 mesh portion was taken as the experimental material. Ascorbic acid (Vc), 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Solarbio Co., Ltd. (Beijing, China). All the reagents were of analytical grade.

Extraction procedure

1.5 g (dry) of *Bletilla striata* powder was placed in a 250 mL Erlenmeyer flask. It was extracted with hot water under the designated liquid-solid ratio, extraction temperature, extraction time, sample immersion time, extraction times and ethanol concentration. After extraction and filtration, the supernatant was concentrated to one quarter of the original volume and then precipitated by the addition of 4 volumes of absolute ethanol overnight at 4 °C. The precipitates were washed by anhydrous alcohol three times. Afterwards, the crude polysaccharides samples were dried using drying cabinet at 35 °C.

Single factor experiment design

The effects of liquid-solid ratio, extraction times, ethanol concentration, sample immersion time, extraction time, and extraction temperature on the yield of BSP were performed using single-factor experimental design, where one factor was changed while others were kept constant. Each experiment was carried out three times in parallel.

Effects of extraction method on extraction rate of BSP

Under the conditions of extraction temperature 50 °C, sample immersion time of 60 min, ethanol concentration of 70%, extraction of 1 time and the ratio of water to raw material of 15 mL/g, ultrasonic and heated reflux were used for extraction. The extraction time was 20, 30 and 40 min, respectively.

Effects of liquid-solid ratio on extraction rate of BSP

Under the conditions of extraction temperature 50 °C, sample immersion time of 60 min, extraction time of 30 min, ethanol concentration of 70% and extraction of 1 time, the ratios of water to raw materials were 15, 20, 25, 30 mL/g, respectively.

Effects of extraction times on extraction rate of BSP

Under the conditions of extraction temperature 50 °C, sample immersion time of 60 min, extraction time of 30 min, ethanol concentration of 70% and the ratio of water to raw material of 15 mL/g, extraction times were 1, 2, 3 and 4, respectively.

Effects of ethanol concentration on extraction rate of BSP

Under the conditions of extraction temperature 50 °C, sample immersion time of 60 min, extraction time of 30 min, extraction 1 time and the ratio of water to raw material of 15 mL/g, ethanol concentrations were 60%, 70%, 80%, 90%, respectively.

Effects of sample immersion time on extraction rate of BSP

Under the conditions of extraction temperature 50 °C, extraction time of 30 min, ethanol concentration of 70%, extraction 1 time and the ratio of water to raw material of 15 mL/g, sample immersion time was 30, 60, 90 and 120 min, respectively.

Effects of extraction time on extraction rate of BSP

Under the conditions of extraction temperature 50 °C, sample immersion time of 60 min, ethanol concentration of 70%, extraction 1 time and the ratio of water to raw material of 15 mL/g, extraction time was 30, 60, 90 and 120 min, respectively.

Effects of extraction temperature on extraction rate of BSP

Under the conditions of sample immersion time of 60 min, extraction time of 30 min, ethanol concentration of 70%, the ratio of water to raw material of 15 mL/g and extraction 1 time, extraction temperature was 50, 60, 70, 80, 90, 95 °C, respectively.

Design of Box-Behnken

Through the single-factor experimental, response surface methodology (RSM) was used to further optimize the experimental parameters. From Table 1, Box-Behnken design (BBD) of BSP was performed with three independent variables extraction temperature (A), extraction time (B), sample immersion time (C) at three levels. The entire design consisted of 17 experimental runs, each of which was carried out in triplicate. The three variables at the three levels were coded as -1, 0 and 1. The total sugar content was measured by the phenol-sulfuric method using glucose as the standard.

Antioxidant Activity *in vitro*

DPPH free radical scavenging activity

The DPPH free radical scavenging activity was detected according to the previous literature with some modifications. Ascorbic acid (Vc) was used as the positive control. Briefly, DPPH ethanol solution (4 mL, 0.0035 mg/mL) was added into 2.00 mL BSP solution at varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL). The mixture was shaken thoroughly and incubated at room temperature in the dark environment for 30 min. The absorbance of the sample was determined at 517 nm. The solution of 2.00 mL deionized water and 4.00 mL absolute ethanol was blank control.^[14-16] The free radical scavenging activity was concluded by the following

equation:

$$\text{Scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

where A_0 is the absorbance of the control, A_1 is the absorbance of the BSP sample, and A_2 is the absorbance of the BSP sample under similar condition to A_1 excepting ethanol instead of the DPPH.

ABTS radical cation scavenging activity

The ABTS radical scavenging of BSP was carried out referring the method of Wang.^[14-16] Vc was used as the positive control. The ABTS radical cation (ABTS^{•+}) was produced via the reaction between ABTS (5 mL, 7 mM) and (NH₄)₂S₂O₈ aqueous solution (1 mL, 15 mM) following 12 h in the dark. The ABTS^{•+} solution was then diluted with deionized water to yield an absorbance of 0.70 at 734 nm. The ABTS^{•+} answer (4 mL) was added to 2 mL BSP solution at varying concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL). After 15 min of reaction, the absorbance was measured at 734 nm. The ABTS^{•+} scavenging activity was concluded by the following equation:

$$\text{Scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100\%$$

where A_0 is the absorbance of the control, A_1 is the absorbance of the BSP sample, and A_2 is the absorbance of all the reaction reagents (except BSP solution).

Statistical analysis

All experiments were performed three times, and SPSS 19.0 was used to analyze data. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the differences among experimental groups. $P < 0.05$ was considered to be statistically significant, and $P < 0.01$ was regarded as highly statistically significant.

Results and Discussion

Single-factor experimental analysis

In this study, the optimization of six key parameters, including liquid-solid ratio, extraction times, ethanol concentration, sample immersion time, extraction time and extraction temperature for improving the yield of BSP was investigated. The effect of extraction method on the yield was showed in Figure 1. The BSP yields of heating reflux extraction were higher than those of ultrasonic extraction at different extraction temperatures. Thus, the heating reflux extraction was selected for extraction of BSP.

Figure 1 The effects of extraction methods on the yield.

The effect of liquid-solid ratio on the yield was showed in Figure 2A. The extraction yield of BSP gradually and slightly increased with the liquid-solid ratio increased from 15 to 25 mL/g, and then it tended to be constant from 25 to 30 mL/g. Large liquid-solid ratio can lead to a slightly increase of the

Table 1 Box-Behnken experiment design and the BSP yield

Run	A/°C	B/min	C/min	Yield of BSP/%
1	90	120	60	46.05
2	95	90	60	41.20
3	85	60	90	42.74
4	90	60	60	44.87
5	95	120	90	43.47
6	85	120	90	45.73
7	90	90	90	48.11
8	95	90	120	42.89
9	90	90	90	48.38
10	85	90	120	44.51
11	90	120	120	46.77
12	90	90	90	48.83
13	90	90	90	47.98
14	90	90	90	48.05
15	85	90	60	43.82
16	95	60	90	40.12
17	90	60	120	46.86

Figure 2 Effects of different experimental variables on the extraction yield of polysaccharides. (A) Liquid-solid ratio, (B) extraction times, (C) ethanol concentration, (D) sample immersion time, (E) extraction time, and (F) extraction and temperature.

extraction yield, and a large amount of extraction solvent can cause waste of energy and increase of workload. Therefore, the liquid-solid ratio of 15 mL/g was selected as the fix parameter for further experiments.

The effect of extraction times on the yield was showed in Figure 2B. The extraction yield of BSP gradually increased with the extraction times from 1 to 3. There was no significant increase when the extraction time was above 2. Therefore, the extraction time of 2 was selected as the fix parameter for further experiments.

The effect of ethanol concentration on the yield was showed in Figure 2C. The extraction yield of BSP gradually and slightly increased with increase of ethanol concentration from 60% to 90%, but it is indistinctive. A large amount of ethanol can cause the waste. It is not important, and the ethanol concentration of 70% was selected as the fix parameter for further experiments.

The effect of sample immersion time on the yield was showed in Figure 2D. The extraction yield of BSP gradually increased with increase of the sample immersion time from 30

Table 2 Analysis of variance (ANOVA) for the quadratic model of BSP extraction

Source	Sum of squares	df	Mean square	F-value	P-value
Model	107.75	9	11.97	20.68	0.0003**
A-X1	10.40	1	10.40	17.96	0.0039**
B-X2	6.90	1	6.90	11.92	0.0107 [#]
C-X3	3.24	1	3.24	5.59	0.0500 [#]
AB	0.032	1	0.032	0.056	0.8198 [#]
AC	0.25	1	0.25	0.43	0.5321 [#]
BC	0.40	1	0.40	0.70	0.4315 [#]
A ²	71.98	1	71.98	124.34	< 0.0001**
B ²	5.12	1	5.12	8.84	0.0207*
C ²	4.31	1	4.31	7.45	0.0293*
Residual	4.05	7	0.58		
Lack of fit	3.51	3	1.17	8.63	0.0321*
Pure error	0.54	4	0.14		
Cor Total	111.81	16			
R ²	0.9638				
Adjusted R ²	0.9172				
Predicted R ²	0.4901				
Adeq precision	12.673				
C.V.%	1.68				

* Significant co-effect ($P < 0.05$). ** High significant co-effect ($P < 0.01$). [#] Not significant ($P > 0.05$).

to 90 min, while it decreased to 120 min. Long large sample immersion time can lead to a slightly increase of extraction yield. Thus, the sample immersion time was selected for further research.

The effect of extraction time on the yield was showed in Figure 2E. The extraction yield of BSP gradually increased with the extraction time increased from 30 to 90 min, while it decreased to 120 min. Long large extraction time can lead to a slightly increase of the extraction yield and it is waste of time. Therefore, the extraction time was selected for further study.

The effect of extraction temperature on the yield was showed in Figure 2F. It is one of the important factors that influence the extraction yield of BSP. The extraction yield of BSP gradually increased with the extraction temperature increased from 50 to 90 °C, while it decreased to 95 °C. Too high extraction temperature is not conducive to the extraction of BSP. Therefore, the extraction temperature was selected for further study.

Fitting the model

Based on the results of single factor experiments, extraction temperature, extraction time and sample immersion time were further investigated using BBD to obtain the optimal extraction conditions. The design matrix and results were shown in Table 1. The response variable Y for extraction yield of BSP can be described by the following equation:

$$Y = 48.25 - 1.14X_1 + 0.93X_2 + 0.64X_3 + 0.090X_1X_2 + 0.25X_1X_3 - 0.32X_2X_3 - 4.13X_1^2 - 1.10X_2^2 - 1.01X_3^2$$

where Y is the BSP yield; X_1 , X_2 and X_3 are the factors of

extraction temperature, extraction time and sample immersion time, respectively.

The analysis of variance (ANOVA) and adequacy of quadratic model were shown in Table 2. The importance of coefficients can be verified by their respective P -values, and smaller P -values signifies a more significant comparing coefficient. In Table 2, the model F -value of 20.68 implies the model in significance. The model P -value was only 0.03% that demonstrates the relapse display for BSP yield was highly significant. Furthermore, the values of determination coefficient ($R^2 = 0.9638$), adjusted determination coefficient (adjusted $R^2 = 0.9172$) and low coefficient of variance (C.V. = 1.68) verified the significance of regression models and the accuracy of experimental data. The linear co-efficient (A), two quadratic coefficients (B^2 and C^2) had a significant effect on the yield ($P < 0.05$), while the other interaction coefficients (AB , AC , BC and A^2) were not significant.

Analysis of response surface

Figure 3 showed 3D response surface plots and their corresponding contour plots about the reciprocal interactions between two independent extraction parameters. As shown in Figures 3A—3D, when extraction time and sample immersion time were designated at level 0, the BSP yield increased firstly with increase of extraction temperature, and then it decreased. When extraction temperature was fixed at level 0, the BSP yield increased with increase of extraction time and sample immersion time. The reason might be that excessive extraction temperature could also cause the degradation of polysaccharides.

Figure 3 Response surface and contour plots for % yield of BSP. (A, B) extraction temperature and extraction time, (C, D) extraction temperature and sample immersion time, (E, F) extraction time and sample immersion time.

Figure 4 Diagnostic plots for the Box-Behnken model adequacy. (A) Normal plot of residuals, (B) plot of internally studentized residuals vs. predicted response, (C) residual vs. run, and (D) predicted vs. actual.

From Figures 3E and 3F, a significant increase of the polysaccharides yield was observed with increase of sample immersion time and extraction time. These two variables had positive reciprocal action on response of extraction yield, however, long extraction time and sample immersion time could not increase the extraction ratio of polysaccharides. The optimum conditions for the BSP yield were obtained as follows:

the extraction time of 90 min, sample immersion time of 90 min, and the extraction temperature of 90 °C. Under these optimal conditions, the maximum extraction yield of BSP was close to the predicted value. These findings indicated that the adequacy of model equation was confirmed, and the optimizing extraction process was suitable for BSP (Figure 4).

Antioxidant activity *in vitro*

DPPH radical is a stable free-radical, which can accept an electron or a hydrogen atom from the antioxidant scavenger molecule to be converted to a more stable DPPH molecule. It is widely used to evaluate the free radical scavenging ability of antioxidants. Studies have shown that polysaccharides exhibited potent antioxidant capacity. Hence, the antioxidant activity of BSP was investigated shown in Figure 5. BSP showed a highest scavenging effect on ABTS^{•+} with scavenging rate of 65.34% at the concentration of 5 mg/mL. Meanwhile, it lowered the scavenging effect on DPPH radical relatively (48.15%), which was correlated positively with increasing concentrations. At the low concentration ranges, the scavenging rate and the concentration of BSP are near linear. However, Vc showed scavenging rates on ABTS^{•+} and DPPH radical of 100%. The results implied that BSP has a slight free radical scavenging ability of antioxidants.

Figure 5 Antioxidant activity *in vitro*. (A) Scavenging activity of Vc to DPPH and ABTS^{•+} from 0 to 50 µg/mL, and (B) scavenging activity of Vc BSP to DPPH and ABTS from 0 to 5 mg/mL.

Conclusion

In this paper, BSP was obtained using conventional hot water extraction and ethanol precipitation from *Bletilla striata*. The maximum yield of polysaccharides was 48.38% under the optimum conditions of liquid-solid ratio of 15 mg/mL, extraction temperature 90 °C, extraction time of 90 h, sample immersion time 90 min, and extraction two times. In addition, the antioxidant assays *in vitro* revealed that BSP has a slight free radical scavenging ability of antioxidants and a scavenging effect of BSP on ABTS^{•+} with scavenging rate of 65.34% at the concentration of 5 mg/mL. However, it lowers the scavenging effect on DPPH radical (48.15%) relatively. The scavenging

effect of BSP on ABTS^{•+} was higher than DPPH radical.

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Author Contributions

X. S. performed the experiments and wrote a draft of the manuscript. W. J. checked the draft, supervised the work and edited the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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References

- [1] Bulpitt, C. J.; Li, Y.; Bulpitt, P. F.; Wang, J. The use of orchids in Chinese medicine. *J. Royal Soc. Med.* **2007**, *100*, 558–563.
- [2] Jiang, F.; Li, M.; Wang, H. Coelonin, an anti-inflammation active component of *Bletilla striata* and Its Potential Mechanism. *Int. J. Mol. Sci.* **2019**, *20*, 4422.
- [3] Jiang, S.; Chen, C. F.; Ma, X. P. Antibacterial stilbenes from the tubers of *Bletilla striata*. *Fitoterapia* **2019**, *138*, 104350.
- [4] Liu, B.; Zhan, Q.; Wu, X. Effect of *Bletilla striata* on the prevention of postoperative peritoneal adhesions in abrasion-induced rat model. *Evid. Based. Complement. Alternat. Med.* **2019**, 9148754.
- [5] Zhou, H.; Jin, Y.; Gu, C. *Bletilla striata* promotes the healing of enterocutaneous fistula: A case report. *Medicine (Baltimore)* **2019**, *98*, e16288.
- [6] Jiang, F.; Li, W.; Huang, Y. Antioxidant, antityrosinase and antitumor activity comparison: the potential utilization of fibrous root part of *Bletilla striata* (Thunb.) Reichb.f. *PLoS One* **2013**, *8*, e58004.
- [7] Qian, C. D.; Jiang, F. S.; Yu, H. S.; Ding, Z. S. Antibacterial Biphenanthrenes from the fibrous roots of *Bletilla striata*. *J. Nat. Prod.* **2015**, *78*, 939–943.
- [8] Shi, Y.; Zhang, B.; Lu, Y. Antiviral activity of phenanthrenes from the medicinal plant *Bletilla striata* against influenza a virus. *BMC Complement. Altern. Med.* **2017**, *17*, 273.
- [9] He, X.; Wang, X.; Fang, J. *Bletilla striata*: Medicinal uses, phytochemistry and pharmacological activities. *J. Ethnopharmacol.* **2017**, *195*, 20–38.
- [10] Chen, Z.; Zhao, Y.; Zhang, M. Structural characterization and antioxidant activity of a new polysaccharide from *Bletilla striata* fibrous roots. *Carbohydr. Polym.* **2020**, *227*, 115362.
- [11] Luo, L.; Liu, Y.; Cai, X. *Bletilla striata* polysaccharides ameliorates lipopolysaccharide-induced injury in intestinal epithelial cells. *Saudi. J. Gastroenterol.* **2019**, *25*, 302–308.
- [12] Wang, Y.; Han, S.; Li, R. Structural characterization and immunological activity of polysaccharides from the tuber of *Bletilla striata*. *Int. J. Biol. Macromol.* **2019**, *122*, 628–635.
- [13] Yue, L.; Wang, W.; Wang, Y. *Bletilla striata* polysaccharide inhibits angiotensin II-induced ROS and inflammation via NOX4 and TLR2 pathways. *Int. J. Biol. Macromol.* **2016**, *89*, 376–388.
- [14] Yu, J.; Hu, M.; Wang, Y. Extraction, partial characterization and

- bioactivity of poly saccharides from *Senecio scandens* Buch.Ham. *Int. J. Biol. Macromol.* **2018**, 109, 535–543.
- [15] Gong, G.; Dang, T.; Deng, Y. Physicochemical properties and biologic activities of polysaccharides from *Lycium barbarum* prepared by fractional precipitation. *Int. J. Biol. Macromol.* **2018**, 109, 611–618.
- [16] Wang, Y.; Jia, J.; Ren, X. Extraction, preliminary characterization and *in vitro* antioxidant activity of polysaccharides from *Oudemansiella dadicata* mushroom. *Int. J. Biol. Macromol.* **2018**, 120, 1760–1769.

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