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ORIGINAL ARTICLE

Polysaccharide extraction optimization, monosaccharide composition, and antioxidant activity analysis of different varieties of *Gastrodia elata Bl* aerial parts

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Abstract

This study investigates the optimal extraction conditions, monosaccharide composition, and antioxidant activity analysis of polysaccharides from the aerial parts of three different varietal varieties of *Gastrodia* (i.e., *G. elata Bl. F. elata, G. elata Bl. F. Viridls MaKino*, and *G. elata Bl. F. Glauca S Chow*). The influence of extraction temperature (30–70 °C), extraction time (15–55 min), and liquid-to-solid ratio (25–65 mL/g) on the yield of polysaccharides was analyzed through single-factor experiments. The response surface methodology was used to optimize the extraction process, and a mathematical model was established to obtain the optimal extraction conditions. The response surface experiment was presented as follows: The optimal extraction conditions for polysaccharides were a liquid-to-solid ratio of 59 mL/g, 56 °C, and 36 min. The polysaccharide yield in *G. elata. Bl. F. Glauca S Chow* aerial parts under these conditions was 10.90%, which was close to the theoretical value calculated by the model (10.64%). Under the optimal conditions, the yields of polysaccharides followed: *G. elata Bl. F. elata* (11.32%) > *G. elata Bl. F. Glauca S Chow* (10.90%) > *G. elata Bl. F. Viridls MalKino* (10.50%). The polysaccharides were mainly composed of 10 monosaccharides, including glucose, rhamnose, mannose, and xylose. However, the content of monosaccharides in polysaccharides of different varieties of *Gastrodia elata Bl* aerial parts varied greatly, with glucose and rhamnose being the highest, both exceeding 20%. The polysaccharides in different varieties of *Gastrodia elata Bl* aerial parts had certain in vitro antioxidant activity. The total reducing power and scavenging rates of 2,2-diphenylpicrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) free radicals increased with the increase of polysaccharide concentration. When the polysaccharide concentration was 1.0 mg/mL, the scavenging rates of DPPH and ABTS free radicals were both over 80%. This study provides a theoretical basis for the further development and utilization of *Gastrodia elata Bl* aerial part resources.

Keywords *Gastrodia elata Bl* aerial part · Polysaccharides · Response surface optimization · Monosaccharide composition · Antioxidant activity

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1 Introduction

Natural products have caused great interests and used for different fields [1–4]. *Gastrodia elata Bl* is a perennial herbaceous plant belonging to the Orchidaceae family. It is also known as "Chijian, Dingfengcao, Shencao, Duyaozhi" and so on. It is mainly distributed in Jilin, Yunnan, Guizhou, Sichuan, Hunan, Hubei, Anhui, Inner Mongolia, and other parts of China. In addition, it is also found in Japan, India, Nepal, the Korean Peninsula, and Siberia [5]. *Gastrodia elata Bl* mainly grows in sparse forests and on the edges of shrubs at elevations of 400–3200 m. The medicinal use of *Gastrodia elata Bl* can be traced back to the "Shennong's Classic of Material Medical," where it is listed as a top-grade herb. It is described as having a pungent and warm taste and is believed to be effective in eliminating evil spirits, poisonous insects, and noxious gases. Long-term consumption is said to promote qi and strength, nourish yin and fat, and prolong life. Clinically, it is mainly used to treat headaches, numbness of limbs, and infantile convulsions. Pharmacological research has shown that *Gastrodia elata Bl* has effects in anti-convulsion, improving learning and memory, and protecting cardiovascular, cerebral, and nervous systems [6–8].

The famous German scientist *Emil Fischer* was one of the first researchers to study sugars, and this research has a history of over a hundred years. In 1993, the USA held the first annual conference on sugar engineering and pointed out that glycobiology, the final major frontier of biochemistry, was rapidly approaching. After proteins and nucleic acids, sugar compounds have become another hotspot in the field of life sciences [9]. Polysaccharides, also known as glycan, are a type of natural high-molecular-weight compounds. They are polar and complex macromolecules composed of aldose or ketose monosaccharides linked together by glycosidic bonds with a degree of polymerization greater than 10. The molecular weight of polysaccharides can range from tens of thousands to millions. Polysaccharides are widely present in various organisms, such as animals, plants, and microorganisms, and have diverse sources [10–13]. In recent years, a large number of studies have found that plant polysaccharides not only have immunomodulatory and anti-tumor biological effects, but also have effects such as reducing blood pressure, anti-coagulation, scavenging free radicals, and delaying aging [14–17]. Moreover, they have little toxicity and side effects on the body. In the research of *Gastrodia elata Bl*, there are also numerous reports on its polysaccharides. Research had shown that *Gastrodia elata Bl* is rich in polysaccharide components [18, 19] and has antioxidant [20] and anti-aging properties [8, 21], neuroprotective activity [22], and immunomodulatory effects [23]. It even plays an effective role in reducing blood lipids, anti-tumor, and antibacterial activities [24].

Gastrodia elata Bl is widely cultivated in China and has a broad distribution. Within the species, numerous variations have emerged, mainly characterized by differences in the appearance of the aerial parts. Based on the distinction in the color of the aerial parts, *Gastrodia* varieties are classified into different variations, including *G. elata Bl. F. elata*, *G. elata Bl. F. viridis MaKino*, and *G. elata Bl. F. Glauca S Chow* [25]. Different varieties of *Gastrodia elata Bl* have different above-ground parts as shown in Fig. 1. There are many studies on *Gastrodia elata Bl* polysaccharides [26], but few reports on polysaccharides in the above-ground parts of *Gastrodia elata Bl*.

The main methods for polysaccharide extraction include hot water extraction, ultrasound-assisted extraction, and enzyme-assisted extraction. Ultrasound-assisted extraction utilizes the mechanical disruption of *Gastrodia* cell wall structures through ultrasonic energy, combined with the thermal effect, to accelerate the solubilization of components from the cells. Compared to other methods,

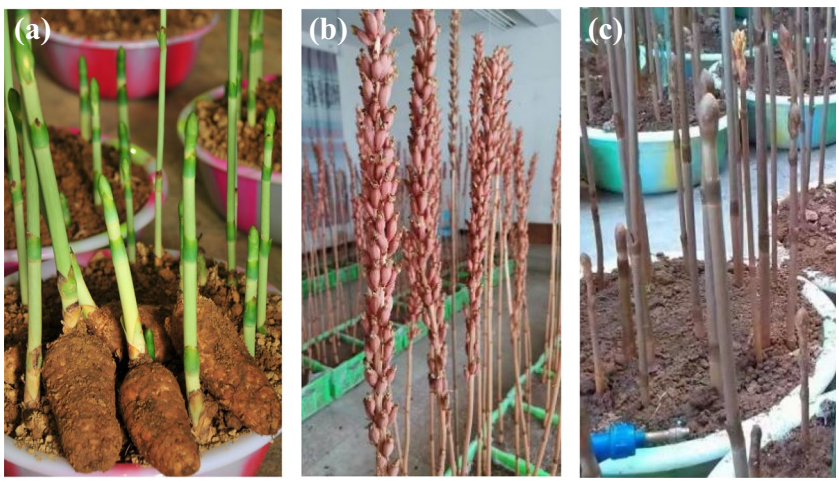


Fig. 1 Different aerial parts of *Gastrodia elata Bl* from different varieties: (**a**) *G. elata Bl. F. Viridls MalKino* aerial parts, (**b**) *G. elata Bl. F. elata* aerial parts, and (**c**) *G. elata Bl. F. Glauca S Chow* aerial parts

ultrasound-assisted extraction offers advantages such as shorter extraction time and higher extraction efficiency, while preserving the polysaccharide activity to the maximum extent. It is considered a favorable polysaccharide extraction method [27–29]. Yunnan Province has abundant *Gastrodia elata Bl* resources, and the above-ground parts of *Gastrodia elata Bl* are by-products generated during *Gastrodia elata Bl* cultivation. With the expansion of the *Gastrodia elata Bl* planting area, the resources of the above-ground parts of *Gastrodia elata Bl* are increasing, but they have not been fully developed and utilized yet [30].

In this study, ultrasound-assisted extraction was used to extract polysaccharides from the above-ground parts of *Gastrodia elata Bl* including *G. elata Bl. F. Glauca S Chow*, *G. elata Bl. F. Viridls MalKino*, and *G. elata Bl. F. elata*. The optimal extraction process of the above-ground parts of *Gastrodia elata Bl* was explored through single-factor and response surface experiments. The monosaccharide composition and content of polysaccharides in the above-ground parts of *Gastrodia elata Bl* were analyzed by pre-column derivatization and high-performance liquid chromatography (HPLC) method. The in vitro antioxidant activity of the above-ground parts of *Gastrodia elata Bl* polysaccharides from different varieties was investigated, including total reducing power, ABTS free radical scavenging rate, and DPPH free radical scavenging rate, in order to provide a theoretical basis for the further development and utilization of the above-ground parts of *Gastrodia elata Bl*.

2 Materials and methods

2.1 Materials and reagents

The materials and reagents used in this study include anhydrous glucose; the above-ground parts of *Gastrodia elata Bl* (i.e., *G. elata Bl. F. Glauca S Chow, G. elata Bl. F. Viridls MalKino, and G. elata Bl. F. elata*); concentrated sulfuric acid (analytical grade); phenol, 3,5-dinitrosalicylic acid, and 2,2-diphenylpicrylhydrazyl (DPPH, purchased from Shanghai Yuan Ye Biotechnology Co., Ltd.); 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS, purchased from Shanghai Yuan Ye Biotechnology Co., Ltd.); and ethanol, potassium ferrocyanide, ferric chloride, potassium persulfate, and concentrated hydrochloric acid (all purchased from China National Pharmaceutical Group Industrial Co., Ltd.). Methanol and trifluoroacetic acid were purchased from ANPEL. Sodium hydroxide and sodium acetate trihydrate were purchased from Sigma. Fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), xylose (Xyl), mannose (Man), fructose (Fru), ribose (Rib), galacturonic acid (Gal-UA), glucuronic acid (Glc-UA), mannuronic acid (Man-UA), and guluronic acid (Gul-UA) were monosaccharide standards and were purchased from Sigma company. Ultra-pure water was in-house prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

3,5-dinitrosalicylic acid (DNS) solution preparation Of 3,5-dinitrosalicylic acid, 6.5 g was added to 325 mL of 2 mol/L NaOH solution and 15 mL of glycerol, which was mixed well and cooled. Finally, distilled water was added to make up the volume to 1000 mL in a brown volumetric flask and set aside.

2.2 Extraction and determination of polysaccharides in *Gastrodia elata Bl*

2.2.1 Polysaccharide extraction process

Gastrodia elata Bl was dried, ground into fine powder, and subjected to ultrasonic extraction with distilled water for three times. The combined filtrate was centrifuged (8000 r/min, 10 min) and then concentrated under reduced pressure. Ethanol was added to the concentrate for precipitation of proteins using the Sevage method [31]. After centrifugation, the supernatant was further treated with ethanol for polysaccharide precipitation. The resulting crude polysaccharide was then freeze-dried to obtain the final product.

2.2.2 Preparation of glucose standard curve

Of glucose stock solution, 0.2, 0.4, 0.6, 0.8, and 1.0 mL were accurately pipetted into 50-mL stoppered test tubes, in which distilled water was added to 2 mL. The solution was shaken well; 1.5 mL of 3, 5-dinitrosalicylic acid (DNS) solution was added and heated in boiling water bath for 5 min. The solution was cooled, made up to 25 mL, and sonicated for 3 min. The absorbance was measured at 540 nm (ultraviolet-visible (UV-Vis) spectrophotometer, UV-2600). The graph was plotted with glucose concentration (C) on the X-axis and absorbance (A) on the Y-axis. The regression equation of the standard curve is as follows: $A = 14.438C + 0.0479$, $R^2 = 0.9982$, indicating a good linearity.

2.2.3 Determination method

Reducing sugar sample solution Two milliliters of crude extract (obtained after centrifugation in the 1.2.1 polysaccharide extraction process) was taken and adjusted to 50 mL with distilled water, which was shaken well to obtain the reducing sugar sample solution.

Total sugar sample solution Five milliliters of the above reducing sugar sample solution was precisely pipetted, in which 5 mL of 6 mol/L hydrochloric acid solution was

added. The mixture was heated in a boiling water bath for 10 min, cooled down to room temperature under running water, neutralized with 40% (w/w) NaOH solution, and finally, the volume was adjusted to 50 mL with distilled water to obtain the total sugar sample solution.

Total sugar and reducing sugar in the aerial parts of *Gastrodia elata Bl* were determined using the DNS colorimetric method. Specifically, 2 mL of the reducing sugar sample and 2 mL of the total sugar sample were precisely transferred into separate 50-mL stoppered test tubes. Then, 1.5 mL of DNS solution was added to each tube, and they were heated in a boiling water bath for 5 min before being cooled in the running water. The volume was adjusted to 25 mL with distilled water, followed by sonication for 3 min. The absorbance was measured at 540 nm, and the results were obtained by fitting the data to the glucose standard curve to obtain ρ_1 and ρ_2. The yield of polysaccharides in the aerial parts of *Gastrodia elata Bl* was determined by the following equations (Eqs. 1–3):

$$m_1 = \rho_1 V d_1 \tag{1}$$

$$m_2 = \rho_2 V d_2 \tag{2}$$

$$X(\%) = \frac{m_2 - m_1}{M} \times 100\% \tag{3}$$

m_1: the mass of reducing sugar; m_2: the total sugar quantity;

M: the mass of *Gastrodia elata Bl*'s aerial part;

ρ_1: the mass concentration of glucose in the solution of the reducing sugar test sample, mg /mL;

ρ_2: the concentration of glucose mass in the total sugar sample solution, mg /mL;

V: the volume of crude extract solution, mL;

d_1 and d_2: dilution factor.

2.3 Experimental design

2.3.1 Single-factor experiment

The single-factor experiments were carried out using the aerial part of *Gastrodia elata Bl* as the raw material, with a fixed ultrasonic frequency of 80 kHz. The main factors investigated were liquid-to-solid ratio (i.e., 25:1, 35:1, 45:1, 55:1, 65:1), ultrasonic temperature (i.e., 30 °C, 40 °C, 50 °C, 60 °C, 70 °C), and ultrasonic time (i.e., 15 min, 25 min, 35 min, 45 min, 55 min) to evaluate their effects on the extraction rate of the aerial parts of *Gastrodia elata Bl*.

Table 1 Factors and levels of Box-Behnken experiment

Factor	Level		
	−1	0	1
Liquid-to-solid ratio (mL/g)	45	55	65
Ultrasonic temperature (°C)	40	50	60
Ultrasonic time (min)	25	35	45

Table 2 Standard curves and linear relationships of monosaccharides

Monosaccharide	Regression equation	R^2	Detection limit (μg/mL)
Fuc	$y = 0.2106x + 0.0825$	0.9984	0.1
Ara	$y = 0.2601x + 0.1345$	0.9966	0.1
Rha	$y = 0.1094x + 0.0252$	0.9984	0.2
Gal	$y = 0.2895x + 0.1123$	0.9965	0.1
Glc	$y = 0.3650x + 0.2541$	0.9977	0.2
Xyl	$y = 0.3163x + 0.1411$	0.9956	0.2
Man	$y = 0.1877x + 0.0019$	0.9956	0.2
Rib	$y = 0.2693x - 0.0848$	0.9991	0.2
Gal-UA	$y = 0.1584x - 0.1171$	0.9958	0.5
Gul-UA	$y = 0.1819x - 0.1569$	0.9952	0.5
Man-UA	$y = 0.0724x - 0.0918$	0.9962	0.5

2.3.2 Response surface optimization design

Based on the results of the single-factor experiments, the liquid-to-solid ratio (i.e., 55 mL/g), ultrasonic temperature (50 °C), and ultrasonic time (35 min) were selected as independent variables. A Box-Behnken design was employed with three factors and three levels to investigate the effect of these variables on the yield of *G. elata Bl. F. Glauca S Chow* aerial part polymer. The factor level design is shown in Table 1. Data analysis was performed using Design Expert 13.0 software.

2.4 Monosaccharide composition and content analysis

2.4.1 Preparation of monosaccharide standard curve

Different gradient concentrations of monosaccharide (i.e., 0.5 μg/mL, 1 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL, 30 μg/mL, 40 μg/mL, and 50 μg/mL) were prepared to serve as reference solution. The quantification was carried out using an external standard method, and a standard curve was drawn by plotting the peak area (y) against the mass concentration (x) of each monosaccharide. The regression equations, linear relationships, and detection limits for each monosaccharide were obtained as shown in Table 2. The linear correlation

coefficients for the 11 monosaccharide reference solutions were all ≥0.9950, indicating good linearity.

2.4.2 Sample preparation and extraction

Approximately 5 mg of polysaccharide sample was hydrolyzed with 1 mL trifluoroacetic acid (2 mol/L) at 121 °C for 120 min in a sealed tube. The sample was washed by methanol, then blow dried, and washed by methanol for 2–3 times. The residue was re-dissolved in deionized water and filtered through 0.22-μm microporous filtering film for measurement.

2.4.3 HPAEC conditions

The sample extracts were analyzed by high-performance anion-exchange chromatography (HPAEC) on a CarboPac PA-20 anion-exchange column (3 by 150 mm; Dionex) using a pulsed amperometric detector (PAD; Dionex ICS 5000 system). The operation was set at the following: flow rate, 0.5 mL/min; injection volume, 5 μL; solvent system, B (0.1 M NaOH, 0.2 M NaAc); and gradient program, 95:5 V/V at 0 min, 80:20 V/V at 30 min, 60:40 V/V at 30.1 min, 60:40 V/V at 45min, 95:5 V/V at 45.1 min, and 95:5 V/V at 60 min. The data were acquired on the ICS5000 (Thermo Scientific) and processed using Chromeleon 7.2 CDS (Thermo Scientific).

2.5 Antioxidant activity measurement

A series of solutions of three different varieties of *Gastrodia elata Bl* polysaccharide were prepared at concentrations of 1 mg/mL, 2.5 mg/mL, 4 mg/mL, 5.5 mg/mL, and 6 mg/mL. The total reducing power, DPPH free radical scavenging rate, and ABTS free radical cation scavenging rate of the samples were measured. The changes of these parameters with the concentration of the sample solutions were analyzed.

2.5.1 Total reducing power

The sample was an antioxidant that can reduce ferricyanide to ferrous ions. Ferrous ions further reacted with ferric chloride to form Prussian blue ($Fe_4[Fe(CN)_6]_3$) with the

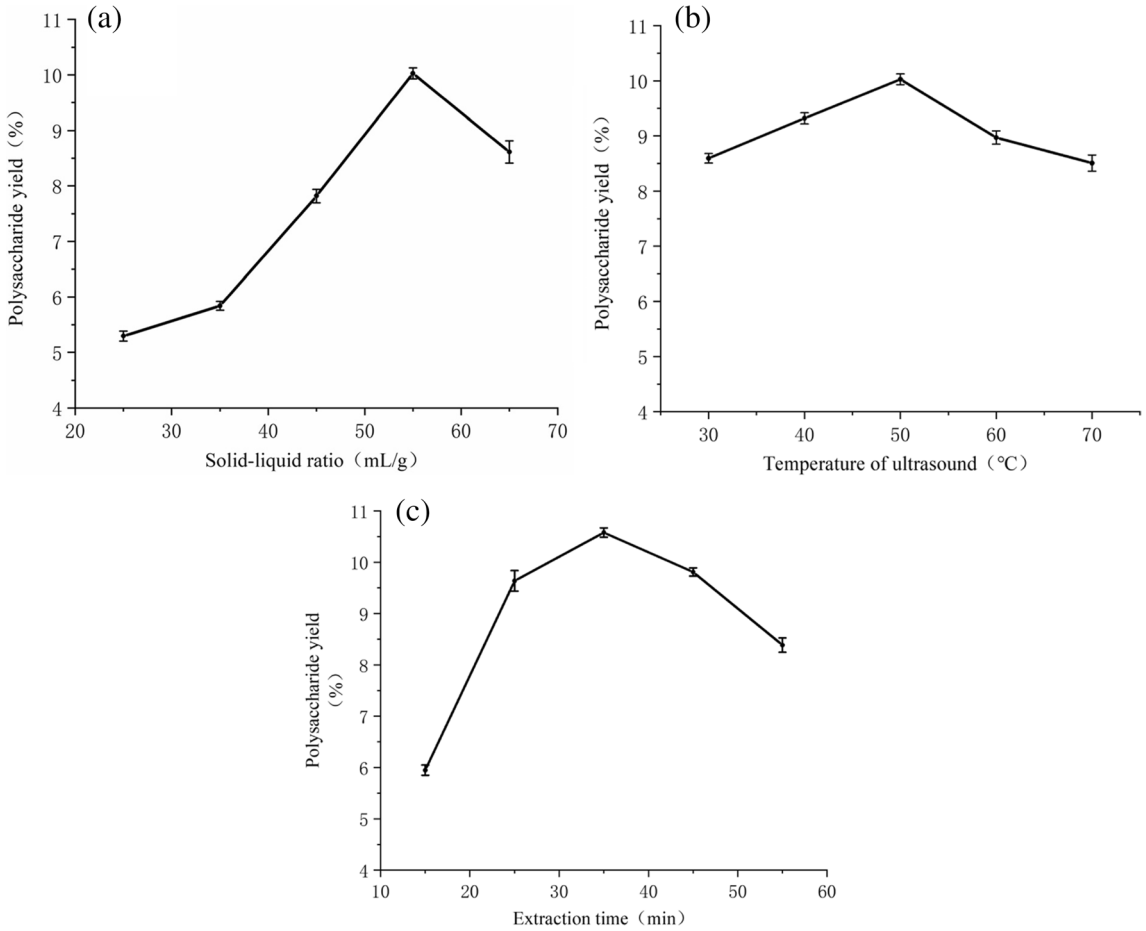


Fig. 2 The effect of different factors on the yield of polysaccharides: (**a**) liquid-to-solid ratio, (**b**) ultrasonic temperature, and (**c**) ultrasonic time

maximum absorbance at 700 nm. Therefore, the level of reduction ability of the sample can be indirectly reflected by the absorbance at 700 nm. The higher the absorbance, the stronger the reduction ability [32].

The measurement can be briefly described as follows: 0.5 mL of phosphate buffer solution (PBS, pH = 6.6) and 0.5 mL of 1% potassium ferricyanide solution were mixed, and then, 0.2 mL of the sample solution was added and mixed well. The mixture was reacted at 50 °C for 20 min, followed by the addition of 0.5 mL trichloroacetic acid (10%). The resulting mixture was centrifuged at 3000 rpm, and 0.5 mL of the supernatant was taken and mixed with 0.5 mL of water and 0.1 mL ferric chloride (0.1%). The mixture was allowed to stand for 10 min, and the absorbance was measured at 700 nm wavelength. Each sample was measured in triplicate.

2.5.2 DPPH radical scavenging activity

The principle is based on the fact that DPPH free radicals have a single electron and exhibit a strong absorption at 517 nm. When a free radical scavenger is present, the electron is paired with the scavenger, causing the absorption of the DPPH free radicals to gradually disappear. The degree of fading is quantitatively related to the number of electrons accepted. The method uses spectrophotometry for rapid quantitative analysis [33–35]. The lower the absorbance, the higher the DPPH free radical scavenging rate is.

The measurement can be briefly described as follows: A standard solution of DPPH with a concentration of 0.04 mg/mL was prepared using anhydrous ethanol as the solvent. Two milliliters of DPPH solution was mixed with 2 mL of sample solution. The absorbance value (A_i) was measured at 517 nm. Each sample was measured in triplicate, and the control group (A_j) and blank group (A_c) were also measured. The DPPH scavenging rate was calculated using the following formula (4). The experiment consisted of sample group, control group, and blank group.

$$K = \left[1 - \frac{A_i - A_j}{A_c}\right] \times 100\% \tag{4}$$

where A_i: 2 mL DPPH + 2 mL sample;
A_j: 2 mL anhydrous ethanol + 2 mL sample;
A_c: 2 mL DPPH + 2 mL anhydrous ethanol.

2.5.3 ABTS radical cation scavenging activity

The principle of this method is to determine the clearing effect of the sample on 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) free radical cation solution by measuring the absorbance at different concentrations of the test substance [36]. The weaker the absorbance, the better the clearing effect on ABTS free radical cation becomes.

Two milliliters of the sample solution was taken, in which 2 mL of ABTS solution was added. After mixing well, the solution remained in the dark for 30 min and the absorbance at 734 nm (A_i) was measured. Each sample was measured in triplicate, and the same was done for the control group (A_j) and the blank group (A_0). The clearance rate was calculated according to the formula (5).

$$K = \left[1 - \frac{A_i - A_j}{A_0}\right] \tag{5}$$

where A_i: 2 mL ABTS + 2 mL sample;
A_j: 2 mL anhydrous ethanol + 2 mL sample;
A_0: 2 mL ABTS + 2 mL anhydrous ethanol.

3 Results and discussion

3.1 Single-factor experiment

Figure 2 a shows the variation of the yield of polysaccharides from the aerial part of *Gastrodia elata Bl* with different liquid-to-solid ratios. As can be seen from Fig. 2, the yield of polysaccharides first increased and then slightly decreased with the increase of liquid-to-solid ratio. When the liquid-to-solid ratio increased from 25 to 55 mL/g, the yield of polysaccharides increased significantly and reached

Table 3 Box-Behnken experimental design and results

Run	X_1 (liquid-to-solid ratio, mL/g)	X_2 (extraction temperature, °C)	X_3 (extraction time, min)	Yield of polysaccharide (%)
1	0 (55)	0 (50)	0 (35)	10.11
2	0 (55)	−1 (40)	1 (45)	7.84
3	−1 (45)	1 (60)	0 (35)	7.84
4	0 (55)	0 (50)	0 (35)	10.48
5	−1 (45)	0 (50)	1 (45)	8.47
6	−1 (45)	−1 (40)	0 (35)	8.44
7	1 (65)	0 (50)	1 (45)	8.61
8	0 (55)	0 (50)	0 (35)	10.26
9	0 (55)	1 (60)	1 (45)	9.89
10	0 (55)	1 (60)	−1 (25)	8.97
11	1 (65)	1 (60)	0 (35)	10.26
12	0 (55)	−1 (40)	−1 (25)	8.45
13	0 (55)	0 (50)	0 (35)	10.84
14	1 (65)	−1 (40)	0 (35)	7.92
15	1 (65)	0 (50)	−1 (25)	8.81
16	0 (55)	0 (50)	0 (35)	10.29
17	−1 (45)	0 (50)	−1 (25)	7.82

the maximum value when the liquid-to-solid ratio was 55 mL/g. With further increase in the liquid-to-solid ratio, the yield of polysaccharides decreased possibly due to the excessive liquid-to-solid ratio, resulting in a decrease in the effective collision area between solvent and solute, leading to a reduction in the yield of polysaccharides [37]. Therefore, the optimal liquid-to-solid ratio was determined to be 55 mL/g.

Figure 2 b shows the variation of polysaccharide yield with ultrasonic temperature. It can be seen that there is no significant change in polysaccharide yield when the ultrasonic temperature is increased from 30 to 70°C, and the yield is distributed between 8 and 10%. When the ultrasonic temperature is 50 °C, the polysaccharide yield is the highest, reaching 10%, and it slightly decreases as the temperature continues to increase. This is because with the increase in temperature, the viscosity of the system decreases, accelerating the mass transfer process. However, when the temperature is too high, it can cause a decrease in surface tension and an increase in vapor pressure inside the air bubbles, leading to a decrease in yield [33, 38]. Therefore, the optimal ultrasonic temperature is 50 °C.

Figure 2 c shows the effect of ultrasonic time on the yield of polysaccharides. As can be seen from the figure, the yield of polysaccharides changes significantly with the change of ultrasonic time. When the ultrasonic time is increased from 15 to 35 min, the yield of polysaccharides increases significantly. When the ultrasonic time is 35 min, the yield is the highest, and it slightly decreases with further extension of ultrasonic time. This is because within a certain time range, with the increase of time, the extraction solution can fully penetrate into the powder of the upper part of *Gastrodia elata Bl*, allowing polysaccharides to be fully dissolved and increasing the yield. However, if the extraction time is too long, polysaccharides may undergo degradation, leading to a decrease in yield. Therefore, the optimal ultrasonic time was chosen as 35 min.

3.2 Response surface optimization experiment

3.2.1 Response surface experimental results

The experimental design and results of response surface methodology for the polysaccharide yield are presented in Table 3. The variance analysis of the fitted quadratic polynomial model is shown in Table 4.

The quadratic regression equation is as follows:

$$Y = 10.4 + 0.38 \times X_1 + 0.54 \times X_2 + 0.093 \times X_3 + 0.74 \times X_1 \times X_2 - 0.21 \times X_1 \times X_3 + 0.38 \times X_2 \times X_3 - 1.07 \times X_1^2 - 0.71 \times X_2^2 - 0.9 \times X_3^2$$

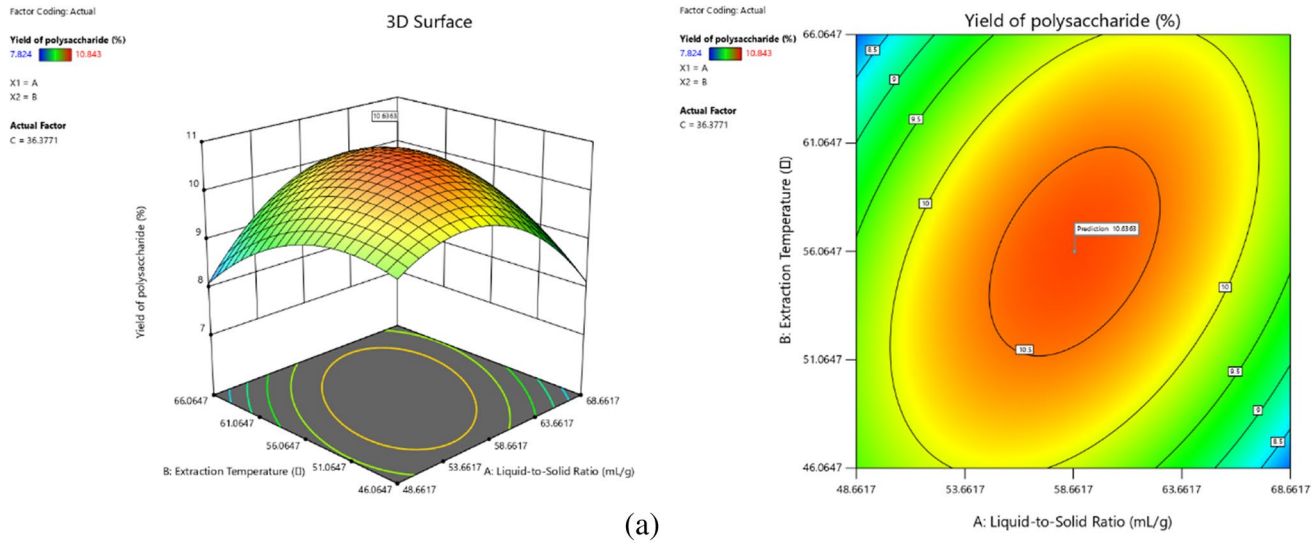
The variance analysis of the regression model shows that the regression model is highly significant with a p-value of less than 0.0001, and the lack-of-fit term is not significant with a p-value of 0.607 (>0.05). The total coefficient of determination R^2 is 0.9739, and the coefficient of variation CV is 2.87%. These parameters indicate that the model has a good fit and low experimental error, so the regression equation is valid and can be used to analyze and detect polysaccharides in the aerial part of *Gastrodia elata Bl*.

Table 4 Analysis of variance (ANOVA) for the regression model

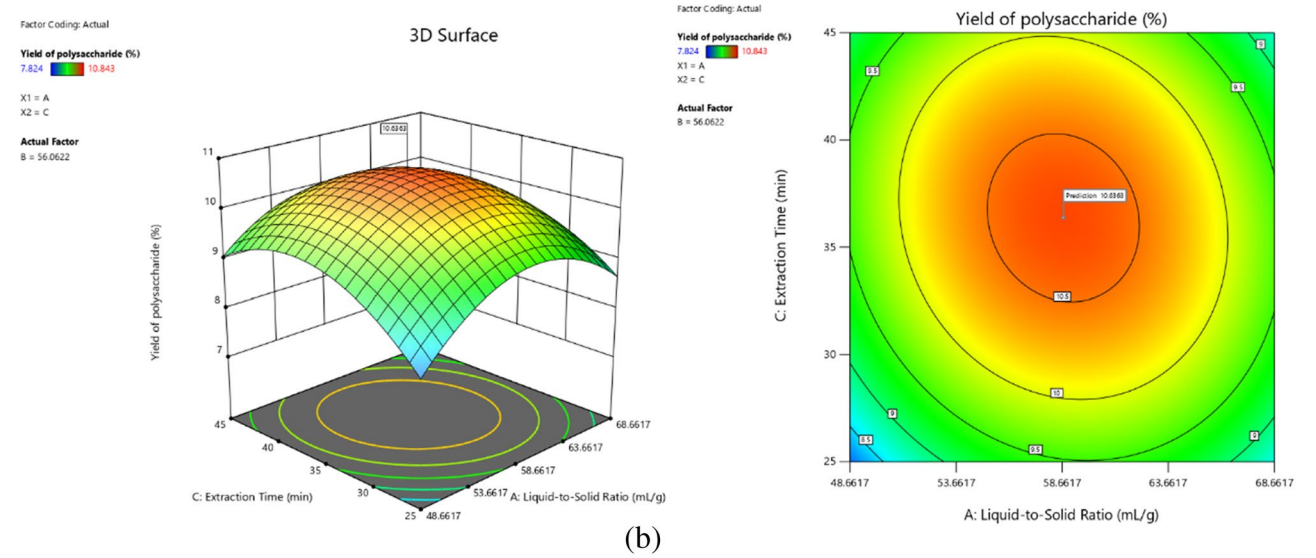
Source	Sum of squares	Df	Mean square	F-value	p-value	Significance
Model	18.00	9	2	29.03	< 0.0001	**
X_1	1.14	1	1.14	16.61	0.0047	*
X_2	2.31	1	2.31	33.56	0.0007	**
X_3	0.069	1	0.069	1.01	0.349	-
X_1X_2	2.17	1	2.17	31.56	0.0008	**
X_1X_3	0.18	1	0.18	2.58	0.1523	-
X_2X_3	0.59	1	0.59	8.49	0.0225	*
X_1^2	4.82	1	4.82	70.01	< 0.0001	**
X_2^2	2.14	1	2.14	31.07	0.0008	**
X_3^2	3.4	1	3.4	49.29	0.0002	**
Residual	0.48	7	0.069			
Lack of fit	0.16	3	0.054	0.68	0.607	-
Pure error	0.32	4	0.08			
Cor. total	18.48	16				
C.V.% = 2.87						
$R^2 = 0.9739$						
$R^2_{adj} = 0.9404$						

"–" means no significant

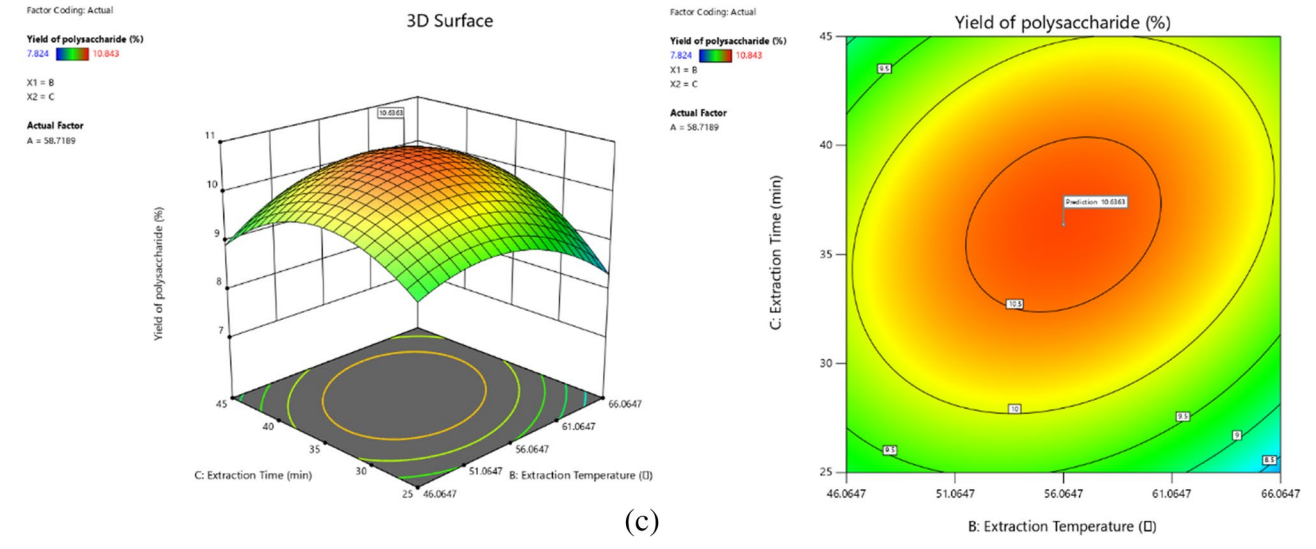
*p-value < 0.05 significant; **p-value < 0.01 highly significant


Factor Coding: Actual
Yield of polysaccharide (%)
7.824
10.843
X1 = A
X2 = B
Actual Factor
C = 36.3771
3D Surface
Yield of polysaccharide (%)
B: Extraction Temperature (°)
A: Liquid-to-Solid Ratio (mL/g)
Yield of polysaccharide (%)
B: Extraction Temperature (°)
A: Liquid-to-Solid Ratio (mL/g)
Prediction 10.6363

(a)


Factor Coding: Actual
Yield of polysaccharide (%)
7.824
10.843
X1 = A
X2 = C
Actual Factor
B = 56.0622
3D Surface
Yield of polysaccharide (%)
C: Extraction Time (min)
A: Liquid-to-Solid Ratio (mL/g)
Yield of polysaccharide (%)
C: Extraction Time (min)
A: Liquid-to-Solid Ratio (mL/g)
Prediction 10.6363

(b)


Factor Coding: Actual
Yield of polysaccharide (%)
7.824
10.843
X1 = B
X2 = C
Actual Factor
A = 58.7189
3D Surface
Yield of polysaccharide (%)
C: Extraction Time (min)
B: Extraction Temperature (°)
Yield of polysaccharide (%)
C: Extraction Time (min)
B: Extraction Temperature (°)
Prediction 10.6363

(c)

◂**Fig. 3** Response surface and contour plots showing the interactive effects of two factors on the polysaccharide yield: (**a**) Liquid-to-solid ratio and temperature, (**b**) liquid-to-solid ratio and time, (**c**) temperature and time

The variance analysis shows that $F_{X1} = 16.61$, $F_{X2} = 33.56$, and $F_{X3} = 1.01$. Among the polysaccharide extraction process parameters, the factors affecting the polysaccharide yield are ranked in the order of importance as follows: temperature > liquid-to-solid ratio > time. X_1 has reached a highly significant level, X_2 has reached a significant level, and the interaction between X_1 and X_2 exists and has reached a highly significant level. However, X_3 and X_1X_3 are not significant.

3.2.2 Response surface analysis

In order to examine the influence of interaction terms on the polysaccharide yield, a dimensionality reduction analysis of the model was conducted while keeping the other factors constant. The response surface and contour plots obtained from Design-Expert 13.0 are shown in Fig. 3. From the contour plots, it can be observed that when two extraction factors are fixed, the polysaccharide yield exhibits a consistent trend of initially increasing and then decreasing as the other influencing factor increases. Figure 3 a illustrates the significant interaction between the liquid-to-solid ratio and extraction temperature at the optimal extraction time of 36 min. Figure 3 b shows that there is no significant interaction between the liquid-to-solid ratio and extraction time at the optimal extraction temperature of 56 °C. Figure 3 c reveals a certain interaction between the extraction time and extraction temperature at the optimal liquid-to-solid ratio of 58.72 mL/g. Among the interaction terms affecting the polysaccharide yield, the liquid-to-solid ratio and extraction time have the least impact, while the liquid-to-solid ratio and extraction temperature have the greatest influence on the polysaccharide yield.

In order to achieve the highest response value of polysaccharide yield, the optimal extraction conditions for polysaccharides from the upper part of *G. elata Bl. F. Glauca S Chow* were obtained through software analysis, which included a liquid-to-solid ratio of 58.72 mL/g, an extraction temperature of 56.06 °C, and an extraction time of 36.38 min. The theoretical maximum polysaccharide extraction rate under these optimal conditions was calculated to be 10.64%.

3.2.3 Optimization process validation and analysis of polysaccharide yield from different varieties of *Gastrodia elata Bl* stem

To verify the reliability of the optimized model, the best extraction conditions for the polysaccharides in the aerial parts of *Gastrodia elata Bl* were revised, resulting in a new optimal condition of a liquid-to-solid ratio of 59 mL/g, a temperature of 56 °C, and a time of 36 min. Three parallel experiments were carried out under these conditions. The polysaccharide yield of *G. elata Bl. F. Glauca S Chow* was found to be 10.9% ± 0.5%, which is close to the theoretical value calculated by the model (10.64%). This indicates that the model parameters obtained by response surface optimization method are reliable. Under the optimal conditions, the polysaccharide yields of *G. elata Bl. F. elata*, *G. elata Bl. F. Glauca S Chow*, and *G. elata Bl. F. Viridls MalKino* were determined to be 11.32% ± 0.8%, 10.90% ± 0.5%, and 10.50% ± 0.7%, respectively. This indicates that there are some differences in the yields of polysaccharides from different species of *Gastrodia elata Bl*, but the differences are not significant.

3.3 Monosaccharide composition and content analysis of polysaccharides

The polysaccharide samples of three different varieties of *Gastrodia elata Bl* were analyzed and determined for their monosaccharide composition and content according to the method described in Section 2.4.1. The results are shown in Table 5.

As shown in Table 5, there are differences in the monosaccharide composition and content of polysaccharides in different varieties of *Gastrodia elata Bl* aerial parts. Polysaccharides are mainly composed of 10 monosaccharides, including glucose, galactose, galacturonic acid, mannose, xylose, and their derivatives. Glucose and galactose have higher content, both exceeding 20%, while galacturonic acid and fucose have lower content (less than 1.20%). The polysaccharides in the aerial parts of *G. elata Bl. F. Viridls MalKino* also contain galacturonic acid. Ribose has the highest content in the polysaccharides of *G. elata Bl. F. Glauca S Chow*, reaching 9.85%, while the content of ribose in the aerial parts of the other two varieties is relatively low. According to related literature, the monosaccharides in *Gastrodia elata Bl* polysaccharides are mainly composed of xylose, rhamnose, glucose, arabinose, and mannose. Compared with *Gastrodia elata Bl* [31], the monosaccharide composition of above-ground part of *Gastrodia elata Bl* polysaccharides is more diverse. Different monosaccharides have different functional properties. Xylose has a potential beneficial effect on skin aging [39], while glucose can promote adhesion between muscle layer and intestinal mucosa, improving constipation [40]. Galacturonic acid is an exciting novel anti-*Pseudomonas aeruginosa* therapeutic agent that targets chronic mucoid *P. aeruginosa* biofilm infection in CF lungs [41]. Mannuronic acid has immunosuppressive properties that can down-regulate Th17 and Th17-related cytokines and promote Th17/Treg imbalance correction,

Table 5 Monosaccharide composition and content analysis

Monosaccharide	*G. elata Bl. F. elata*/%	*G. elata Bl. F. Glauca S Chow*/%	*G. elata Bl. F. Viridls Mal-Kino*/%
Fuc	0.76	0.90	1.10
Ara	8.17	4.10	9.65
Rha	6.20	4.37	4.18
Gal	28.89	26.29	30.78
Glc	29.16	37.32	21.84
Xyl	2.05	1.32	2.43
Man	9.81	4.69	9.30
Rib	0.77	9.85	1.19
Gal-UA	13.85	10.97	14.76
Gul-UA	0.35	0.20	1.17
Man-UA	-	-	0.60

which can treat ankylosing spondylitis [42]. Ribose can regulate the production of oxygen-free radicals during and, after exercise, promote the recovery of ATP and TAN levels in myocardium or skeletal muscle after ischemia or high-intensity exercise [43].

3.4 In vitro antioxidant activity evaluation

The total reducing power of different varieties of above-ground parts of *Gastrodia elata Bl* polysaccharides is shown in Fig. 4a. It can be seen from the figure that the total reducing power of above-ground polysaccharides of *Gastrodia elata Bl* increases with increasing the polysaccharide concentration. At the same concentration, the total reducing power of above-ground polysaccharides of *G. elata Bl. F. Glauca S Chow* is the highest, followed by that of *G. elata*

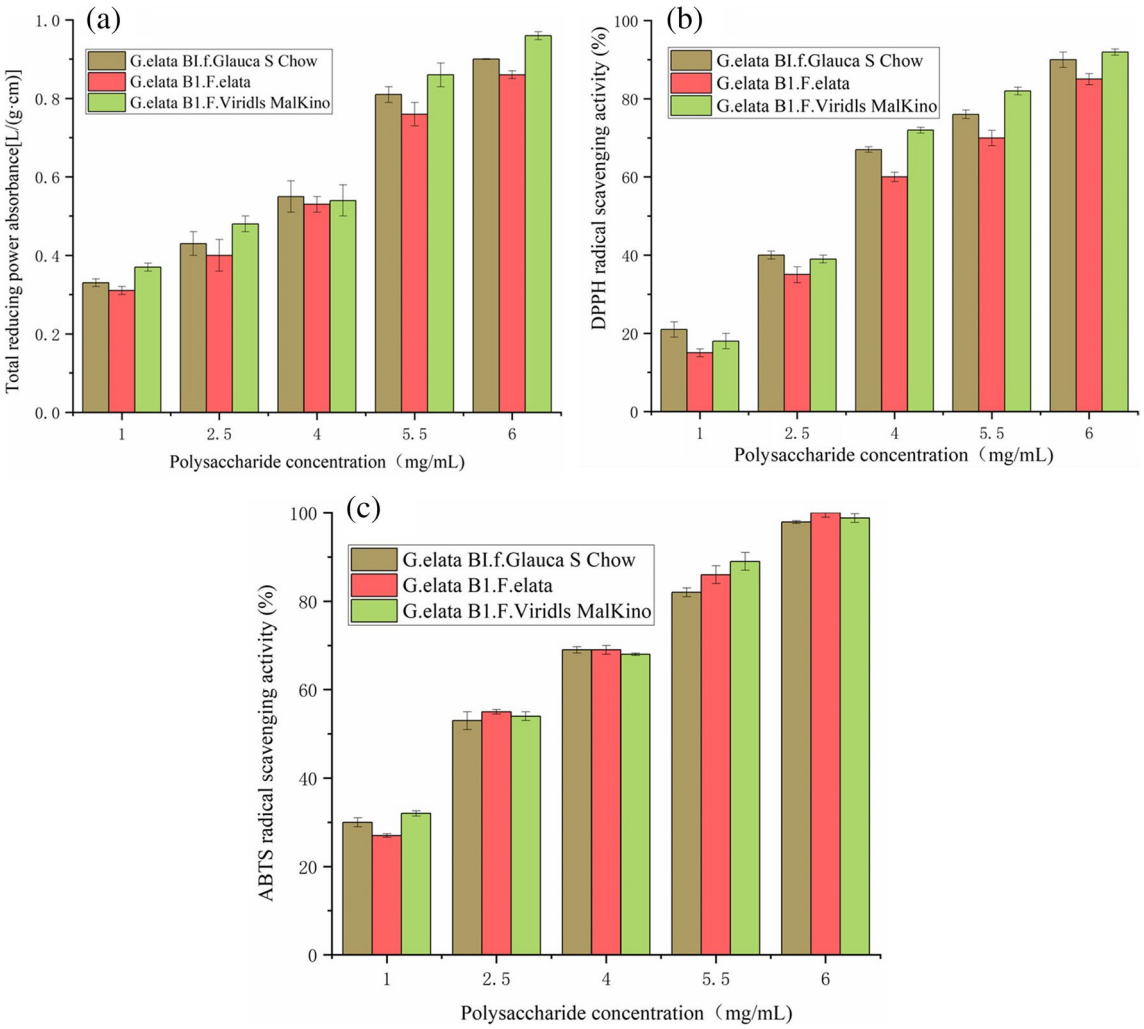


Fig. 4 In vitro antioxidant activity comparison of polysaccharides from different varieties of *Gastrodia elata Bl:* (**a**) total reducing power; (**b**) DPPH radical scavenging activity, and (**c**) ABTS radical scavenging activity

Bl. F. elata and the total reducing power of above-ground polysaccharides of *G.elata Bl. F. Viridls MalKino* is slightly lower.

Figure 4 b shows the scavenging rate of DPPH radicals by different concentrations of above-ground polysaccharides of different varieties of *Gastrodia elata Bl*. The graph shows that as the concentration of polysaccharides from the aerial parts of *Gastrodia elata Bl* increases from 1 to 6 mg/mL, the DPPH radical scavenging activity increases significantly. However, as the concentration continues to increase, the DPPH scavenging activity increases slowly. At the same concentration, the DPPH radical scavenging activity of polysaccharides from different varieties of *Gastrodia elata Bl* aerial parts follows the order: *G. elata Bl. F. Glauca S Chow* > *G.elata Bl. F. Viridls MalKino* > *G. elata Bl. F. elata.*

Figure 4 c shows the ABTS radical scavenging activity of polysaccharides from different varieties of *Gastrodia elata Bl* aerial part at different concentrations. A higher scavenging rate indicates a stronger ability to clear ABTS radicals. As shown in the figure, the ABTS radical scavenging rate is positively correlated with the polysaccharide concentration, meaning that the scavenging rate increases with increasing the polysaccharide concentration. At the same concentration, the three types of *Gastrodia elata Bl* aerial part polysaccharides have similar ABTS radical scavenging rates.

Wang et al. [33] investigated the antioxidant activity of *G. elata Bl. F. Glauca S Chow* polysaccharides and found that the maximum clearance rates of DPPH and ABTS radicals were about 50% when the polysaccharide concentration was 5 mg/mL, while a concentration of 2.5 mg/mL of *G. elata Bl. F. Glauca S Chow* aerial part polysaccharides was sufficient to achieve 50% clearance of ABTS radicals. When the concentration of *G. elata Bl. F. Glauca S Chow* aerial part polysaccharides was 5.5 mg/mL, the clearance rates of DPPH and ABTS radicals were both over 80%. These findings suggest that the antioxidant activity of *Gastrodia elata Bl* aerial part polysaccharides is superior to that of *Gastrodia elata Bl* polysaccharides, possibly due to different compositions of these two polysaccharides [44].

4 Conclusion

The present study employed an ultrasound-assisted extraction method to extract polysaccharides from the aerial part of *Gastrodia elata Bl*. The total sugar and reducing sugar contents were determined by the DNS method to calculate the polysaccharide yield. The effects of the liquid-to-solid ratio, ultrasonic time, and ultrasonic temperature on the yield of polysaccharides from the aerial part of *Gastrodia elata Bl* were analyzed by a single-factor experiment. A response surface design optimization experiment was conducted to determine the order of the primary and secondary factors affecting the polysaccharide yield, which were found to be the liquid-to-solid ratio > time > temperature. The liquid-to-solid ratio and extraction temperature reached a highly significant level. The optimal extraction conditions for the polysaccharides from the aerial part of *Gastrodia elata Bl* were determined to be a liquid-to-solid ratio of 59 mL/mg, a temperature of 56 °C, and a time of 36 min. Under the optimal extraction conditions, the yield of polysaccharides from the aerial part of *G. elata Bl. F. Glauca S Chow* was 10.90%, which is close to the theoretical value of 10.64% predicted by the model obtained from the response surface design. This indicates that the model parameters obtained from the response surface design are reliable. The yields of polysaccharides from the aerial parts of *G. elata Bl. F. elata* and *G. elata Bl. F. Viridls MalKino* were 11.32% and 10.50%, respectively, under the optimal extraction conditions. Through analysis of the monosaccharide composition and content in different varieties of *Gastrodia elata Bl* aerial parts polysaccharides, it was found that it is mainly composed of 10 monosaccharides, with glucose and rhamnose as the main constituents, accounting for 20–40% of the total composition. The monosaccharide content varies greatly among different varieties of *Gastrodia elata Bl* aerial parts polysaccharides, with the ribose content in the aerial parts polysaccharides of *G. elata Bl. F. Glauca S Chow* reaching 9.85%, which is much higher than that of *G. elata Bl. F. Viridls MalKino* (0.77%) and *G. elata Bl. F. elata* (1.19%). The aerial parts of polysaccharides of *G. elata Bl. F. Viridls MalKino* also contain arabinogalactan. The monosaccharide composition in *Gastrodia elata Bl* aerial parts polysaccharides is more diverse than that in *Gastrodia elata Bl* polysaccharides. The study on the in vitro antioxidant activity showed that there were some differences in the antioxidant activity of different varieties of *Gastrodia elata Bl* aerial parts polysaccharides. The total reducing power and the scavenging ability on DPPH and ABTS free radicals increased with the increase of polysaccharide concentration. Under the same concentration of polysaccharide solution, the DPPH radical scavenging rate was the highest in the polysaccharides from the aerial parts of *G. elata Bl. F. Glauca S Chow*, followed by those from the aerial parts of *G. elata Bl. F. Viridls MalKino* and *G. elata Bl. F. elata*, while the ABTS radical scavenging rate was similar among different varieties. The comparison with the literature shows that the antioxidant activity of the polysaccharides extracted from the aerial parts of *Gastrodia elata Bl* is significantly better than that of the polysaccharides extracted from the whole plant. This suggests that there are significant structural differences between the polysaccharides extracted from the aerial parts and the whole plant of *Gastrodia elata Bl*. This study provides a theoretical basis for further research on the polysaccharides extracted from the aerial parts of *Gastrodia elata Bl* and provides a scientific basis for the development of medicinal value of the aerial parts of *Gastrodia elata Bl*.

Author contributions Chunjiang Du: investigation, writing — review and editing. Chengshan Wei: investigation, writing — review and editing. Hassan Algadi: software, validation. Xiangyi Liu: methodology, software, visualization, writing — original draft. Ying Hou: software, visualization, writing — original draft. Handong Li: data curation, writing — review and editing, formal analysis. Jincheng Fan: data curation, formal analysis. Man Vir Singh: data curation, writing — review and editing. Yunxian Li: data curation, writing — review and editing, formal analysis. Juan Xu: supervision, funding acquisition, conceptualization, project administration. Zhanhu Guo: supervision, conceptualization, data curation, writing — review and editing.

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Data availability Data will be made available on request.

Declarations

Ethics declarations Not available.

Competing interests The authors declare no competing interests.

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