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Extraction optimisation, purification, characterisation, antibacterial, and antioxidant activity of polysaccharide from *Platycodon grandiflorus* leaves

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Abstract

Platycodon platycodon is a traditional Chinese medicine homologous food, which is rich in stems and leaves, but mostly discarded. In the present work, the extraction process of polysaccharide from *P. grandiflorus* leaves (PGLP) was optimised. After isolation and purification, the antibacterial and antioxidant activities of the polysaccharide were studied. The extraction rate was highest under the conditions of ultrasonic power 280 W, solid-to-solvent ratio 1:22 (w/v), extraction temperature 79°C, and extraction time 3.5 h, reaching 10.11%, which was consistent with the model predicted value. The crude polysaccharide was isolated and purified by DEAE-52 cellulose chromatography and Sephadex G-100 chromatography column, and two components PGLP-2-1 and PGLP-4-1 were obtained. UV-Vis, FT-IR, and XRD analyses showed that PGLP-2-1 and PGLP-4-1 had typical characteristics of polysaccharides. PGLP-2-1 had the highest antibacterial activity against *Staphylococcus aureus*, and the inhibitory zone diameter reached 17.46 mm; in addition, it had weaker antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, and *Bacillus subtilis*. PGLP-4-1 had a weak antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, and *Staphylococcus aureus*, and the inhibitory zone diameters were 12.24, 11.35, and 12.52 mm, respectively. PGLP-2-1 had strong scavenging ability on superoxide anion, hydroxyl, DPPH, and ABTS free radicals, and the highest scavenging rate reached 86.68, 80.96, 74.82, and 78.88%, respectively. PGLP-4-1 also had strong scavenging ability on superoxide anion, hydroxyl, DPPH, and ABTS free radicals; and the highest scavenging rate reached 75.04, 72.23, 60.56, and 50.25%, respectively. The results indicated that PGLP-2-1 and PGLP-4-1 had strong antioxidant activity, and the antioxidant activity of PGLP-2-1 was higher than PGLP-4-1. The present work provided a theoretical basis for the comprehensive development and utilisation of *P. platycodon* leaves.

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Introduction

Radix platycodonis is a dried root of *Platycodon grandiflorus*, which is first published in "Shen Nong's Herbal Classic". It has the effects of ventilating the lungs, clearing the throat, and expelling phlegm, and it is effective in treating cough, sore throat, and spitting of the lungs (Zhang *et al*., 2015). *Platycodon grandiflorus* is a traditional Chinese medicine homologous food, and its tender roots, stems, and leaves are all edible (Ahn *et al*., 1996). Modern researchers have found that it is not only rich in saponins, polysaccharides, terpenoids, flavones, polyphenols, steroids, and polyacetylene, but also contains a variety of amino acids, unsaturated fatty acids, and various essential trace elements, such as magnesium and zinc (Mazol *et al*., 2004; Liu *et al*., 2006; Choi *et al*., 2008). *Radix platycodonis* polysaccharide has physiological functions such as hypoglycaemic, antioxidant, anticancer, anti-fatigue, and strengthening immunity, and is widely used in medicine and health food (Park *et al*., 2014; Wang *et al*., 2019).

Roots are the traditional medicinal part of *P. grandiflorum*, while the above-ground parts are rich in stems and leaves, and mostly discarded. Studies had shown that *P. grandiflorum* stem was rich in flavonoids, and its extracts had significant anti-inflammatory and expectorant effects (Inada *et al*., 1992). Wang *et al*. (2017a) found that the content of triterpenoid saponins, flavonoids, and steroids in stems and leaves of *P. grandiflorum* was abundant,

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and extracts of leaves had significant antidepressant activity.

At present, most of the research on *P. grandiflorum* are focused on roots, but few on leaves, and there are even fewer reports on polysaccharide from *P. grandiflorum* leaves. In the present work, the extraction process of polysaccharide from *P. grandiflorum* leaves was optimised by response surface methodology, the structure was characterised by ultraviolet-visible (UV-Vis), Fourier-transform infrared (FT-IR), X-ray diffraction (XRD), and its antibacterial and antioxidant activities were studied to provide a theory gist for the comprehensive utilisation of *P. grandiflorum* leaves.

Materials and methods

Materials

Platycodon grandiflorum leaves were gathered from the mountains of Luoyang, Henan Province, China, which were then sieved using a 40-mesh screen after drying and crushing. The tested strains included Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Pastevula mulfocida*), which were provided by the College of Food and Bioengineering, Henan University of Science and Technology, China.

Extraction of polysaccharide from Platycodon grandiflorum leaves

The extraction process was done by weighing the ground *P. grandiflorum* leaves, putting them in a Soxhlet extractor, degreasing with petroleum ether, and then drying them out. An appropriate amount of distilled water was added as the extraction medium and mixed well. Later, it was ultrasonic crushed, heated in a water bath at a certain time, centrifuged at 8,000 *g* for 10 min, and the supernatant collected. Absolute ethanol was added three times to the supernatant, and incubated overnight. The precipitation was taken after centrifuging at 8,000 *g* for 15 min. The Sevage method was used to remove protein, and the H_2O_2 method was used to remove pigment, and then vacuum freeze-drying was performed to obtain the crude polysaccharide.

Determination of polysaccharide extraction rate

The concentration of polysaccharide was determined by phenol-sulphuric acid colorimetry (Babamoradi *et al*., 2018). The polysaccharide concentration was calculated using a linear equation: $y = 11.916x - 0.0453$, $R^2 = 0.9969$ (using glucose as the standard curve to get the relationship between sugar concentration and absorbance value). The polysaccharide extraction rate was then calculated using Eq. 1:

$$Polysaccharide\ extraction\ rate\ (\%) = \frac{Polysaccharide\ concentration \times Polysaccharide\ solution\ volume}{Dry\ weight\ of\ sample} \times 100\% \quad \text{(Eq. 1)}$$

Single factor test

The effects of ultrasonic power (100, 200, 300, 400, 500, and 600 W), solid-to-solvent ratio (1:10, 1:20, 1:30, 1:40, and 1:50 (w/v)), extraction temperature (40, 50, 60, 70, 80, and 90°C), and extraction time (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 h) on the extraction effect of polysaccharide from *P. grandiflorum* leaves were then investigated.

Response surface test design

Based on single-factor test results, the four-factor and three-level central composite design (CCD) was performed: ultrasonic power (X_1), solid-to-solvent ratio (X_2), extraction temperature (X_3), and extraction time (X_4) (Table 1).

Separation and purification of polysaccharide

The crude polysaccharide from *P. grandiflorum* leaves was prepared as a 5 mg/mL solution, and the DEAE-52 chromatography column (2.6 × 30 cm) was used for chromatography. The sample quantity was 10 mL, and the flow rate was 1.25 mL/min. Distilled water, and 0.10, 0.20, 0.30, and 0.40 mol/L NaCl solutions were used for gradient elution. One tube was collected every 5 mL by the automatic fraction collector. The phenol-sulphuric acid method was used to measure the absorbance at 490 nm, and the elution curve was plotted. The peak eluate samples were combined, dialysed against deionised water for 2 d, and freeze-dried in a vacuum.

Lyophilised groups collected from DEAE-52 column chromatography were distributed to make 5 mg/mL solutions, and 2 mL was loaded. Sephadex G-100 column was used for chromatography, and the flow rate was 0.5 mL/min with distilled water as eluent. The eluate was collected by an automatic fraction collector, and the absorbance was measured to plot an elution curve. The peak eluate samples were combined, dialysed, and vacuum freeze-dried to obtain refined polysaccharide.

Table 1. Scheme and results of central composite design.

Run	Factor and level				Extraction rate of polysaccharide (%)
	X_1 Ultrasonic power	X_2 Solid to solvent ratio	X_3 Extraction temperature	X_4 Extraction time	
1	300	1:40	70	2.5	8.19 ± 0.33
2	250	1:25	75	3.0	9.03 ± 0.35
3	350	1:25	75	2.0	7.18 ± 0.28
4	350	1:35	75	3.0	7.13 ± 0.29
5	350	1:25	65	3.0	7.95 ± 0.31
6	300	1:30	70	1.5	7.96 ± 0.30
7	350	1:35	75	2.0	6.13 ± 0.23
8	300	1:30	70	2.5	9.16 ± 0.35
9	250	1:35	65	2.0	7.93 ± 0.32
10	300	1:30	70	2.5	8.19 ± 0.33
11	300	1:30	80	2.5	8.58 ± 0.33
12	250	1:35	75	3.0	8.17 ± 0.29
13	250	1:25	75	2.0	8.46 ± 0.34
14	400	1:30	70	2.5	4.98 ± 0.19
15	250	1:35	75	2.0	7.60 ± 0.27
16	350	1:25	75	3.0	8.09 ± 0.31
17	300	1:30	60	2.5	7.22 ± 0.27
18	350	1:35	65	2.0	7.09 ± 0.26
19	350	1:25	65	2.0	7.21 ± 0.28
20	350	1:35	65	3.0	8.31 ± 0.31
21	200	1:30	70	2.5	7.30 ± 0.29
22	300	1:30	70	3.5	9.45 ± 0.37
23	250	1:35	65	3.0	8.41 ± 0.33
24	300	1:30	70	2.5	8.79 ± 0.34
25	250	1:25	65	3.0	8.11 ± 0.31
26	300	1:20	70	2.5	9.02 ± 0.37
27	250	1:25	65	2.0	7.05 ± 0.27

UV-Vis spectroscopy analysis

UV-Vis spectroscopy analysis was performed according to Sun *et al*. (2023), where 20 mg of sample was weighed and configured to 1 mg/mL solution with distilled water. Ultraviolet-visible spectrophotometry was used to scan in 200 - 500 nm, with a wavelength interval of 5 nm.

FT-IR analysis

FT-IR analysis was performed according to Sun *et al*. (2023), where 2 mg of sample was weighed and mixed with potassium bromide (KBr) at a ratio of 1:100 for grinding. Using KBr as background, the detection was carried out in the range of 4000 - 400 cm^{-1} wavelength.

XRD analysis

XRD analysis was performed according to Sun *et al*. (2023). An appropriate amount of samples were taken on a glass carrier plate, crushed and compacted, and placed on the loading platform of XRD with Cu Kα radiation, 40 kV voltage, 30 mA current, and 4 (°)/min scanning rate.

Bacteriostatic experiment

The polysaccharide was dissolved to 50 mg/mL with sterile distilled water, filtration sterilisation, split charging, and cryopreservation. The test bacteria were inoculated on the bacterial medium, and incubated in a constant temperature incubator at 37°C for 1 d. The activated bacteria were picked up by an inoculating loop, and placed in sterilised distilled water, and then mixed to make a bacterial suspension.

The filter paper method (Sun *et al*., 2017) was used for the bacteriostatic test: the sterilised round filter paper (6 mm Ø) was dried and immersed in the prepared polysaccharide solution for 12 h. Then, the prepared various bacterial suspensions (200 μL) were placed on the solid medium surface, and spread evenly. The soaked filter paper was pasted on the medium surface with sterilised forceps, and incubated in a constant temperature incubator at 37°C for 18~24 h. The inhibition zone was observed, and its diameter was measured. Ampicillin was used as positive control, and sterile distilled water as blank control.

Determination of superoxide anion radical scavenging ability

Following the method of Yuan *et al*. (2014), 0.1 mL of polysaccharide solution with different mass concentrations (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL) was taken, and 2.8 mL of Tris-HCl buffer solution (0.05 mol/L) and 0.1 mL of pyrogallol (3 mmol/L) were added. The control group was distilled water, while the blank group was 2.8 mL of Tris-HCl buffer solution, 0.1 mL of distilled water, and 0.1 mL of HCl (0.01 mol/L). The positive control was vitamin C (Vc). After reaction for 30 s, the absorbance was determined at 320 nm every 30 s, and the reaction was performed for 5 min. The superoxide anion radical scavenging ability was then calculated using Eq. 2:

$$Superoxide\ anion\ radical\ scavenging\ rate\ (\%) = \left(\frac{Acontrol - Asample}{Acontrol}\right) \times 100\% \quad \text{(Eq. 2)}$$

where, $A_{control}$ = control group absorbance, and A_{sample} = sample solution absorbance.

Determination of hydroxyl radical scavenging ability

Following the method of Yuan *et al*. (2014), 0.4 mL of pH 7.4 PBS, 0.25 mL of redistilled water, 0.5 mL of $FeSO_4$ (7.5 mmol/L), and 0.15 mL of phenanthroline solution (5 mmol/L) were added into the test tube, and mixed evenly. Then, 1.0 mL of polysaccharide solution and 0.1 mL of 1% hydrogen peroxide solution was added. The absorbance was measured at 536 nm after incubation in a water bath at 37°C for 60 min. The Vc of corresponding concentration was used as the positive control, damaged tube was the tube with no sample, the control was the tube with no sample and hydrogen peroxide, and the blank tube was the tube with no hydrogen peroxide. Each sample was measured three times, and the mean value was taken. The hydroxyl radical scavenging rate was then calculated using Eq. 3:

$$Hydroxyl\ radical\ scavenging\ rate\ (\%) = \frac{Asample - Adamaged}{Acontrol - Ablank} \times 100\% \quad \text{(Eq. 3)}$$

where, A_{sample} = sample tube absorbance, $A_{damaged}$ = damaged tube absorbance, $A_{control}$ = control tube absorbance, and A_{blank} = blank tube absorbance.

Determination of DPPH free radical scavenging ability

Following the method of Sun *et al*. (2023), 2 mL of sample solution with different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL) was added into the test tube together with 2 mL of 0.1 mmol/L DPPH-ethanol solution, evenly mixed, and reacted at room temperature and away from light for 30 min. Anhydrous ethanol was used as blank control, and Vc as positive control. The absorbance was measured at 517 nm, and the DPPH radical scavenging rate was then calculated using Eq. 4:

$$DPPH\ free\ radical\ clearance(\%) = \left[\frac{1-(A_1-A_2)}{A_0}\right] \times 100\% \quad \text{(Eq. 4)}$$

where, A_1 = sample absorbance, A_2 = sample background absorbance, and A_0 = blank control absorbance.

Determination of ABTS free radical scavenging ability

Following the of Sun *et al*. (2023), 1 mL of sample solution with different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL) was taken, and 4 mL of ABTS was added, and mixed well to make the reaction complete. Reacting at room temperature and away from light for 6 min, the absorbance value was measured at 734 nm, and Vc was used as a positive control. The ABTS free radical clearance was then calculated using Eq. 5:

$$ABTS\ free\ radical\ clearance\ (\%) = \left[\frac{1-(A_1-A_2)}{A_0}\right] \times 100 \quad \text{(Eq. 5)}$$

where, A_1 = sample absorbance, A_2 = absorbance of ABTS replaced by anhydrous ethanol, and A_0 = water absorbance.

Statistical analysis

The main experiments were performed and repeated at least three times unless otherwise stated. The experimental results were presented as mean ± SD (standard deviation). Statistical analysis was analysed using SPSS 18.0.

Results and discussion

Single-factor test

With the increase in ultrasonic power, the extraction rate of polysaccharide first increased, and then decreased (Figure 1a). When the ultrasonic power was 300 W, the extraction rate of polysaccharide reached the highest at 6.22%, and then decreased gradually. Due to the mechanical and cavitation effect of ultrasonic waves, the cell wall and membrane structure of *P. grandiflorum* leaves would be destroyed, so that polysaccharide could be dissolved more easily. Moreover, the more fully the cell wall was destroyed with the increase of ultrasonic power, the more fully the polysaccharide would be dissolved. However, high ultrasonic power might change the structure of polysaccharide, and cause reactions between polysaccharide and other components, leading to a decrease in the polysaccharide extraction rate. Therefore, the ultrasonic power was chosen to be 300 W.

The extraction rate of polysaccharide increased gradually with the decrease in the solid-to-solvent ratio (Figure 1b). When solid-to-solvent ratio was 1:30 (w/v), the extraction rate was 6.25%, and then gradually stabilised. This was probably because the total amount of dissolved polysaccharide in *P. grandiflorum* leaves would increase with the increase in extract solution amount. In addition, the concentration of protein in the whole extraction system would decrease with the increase in extract solution amount, which would decrease the adsorption effect of protein deposition on polysaccharide and the loss of polysaccharide

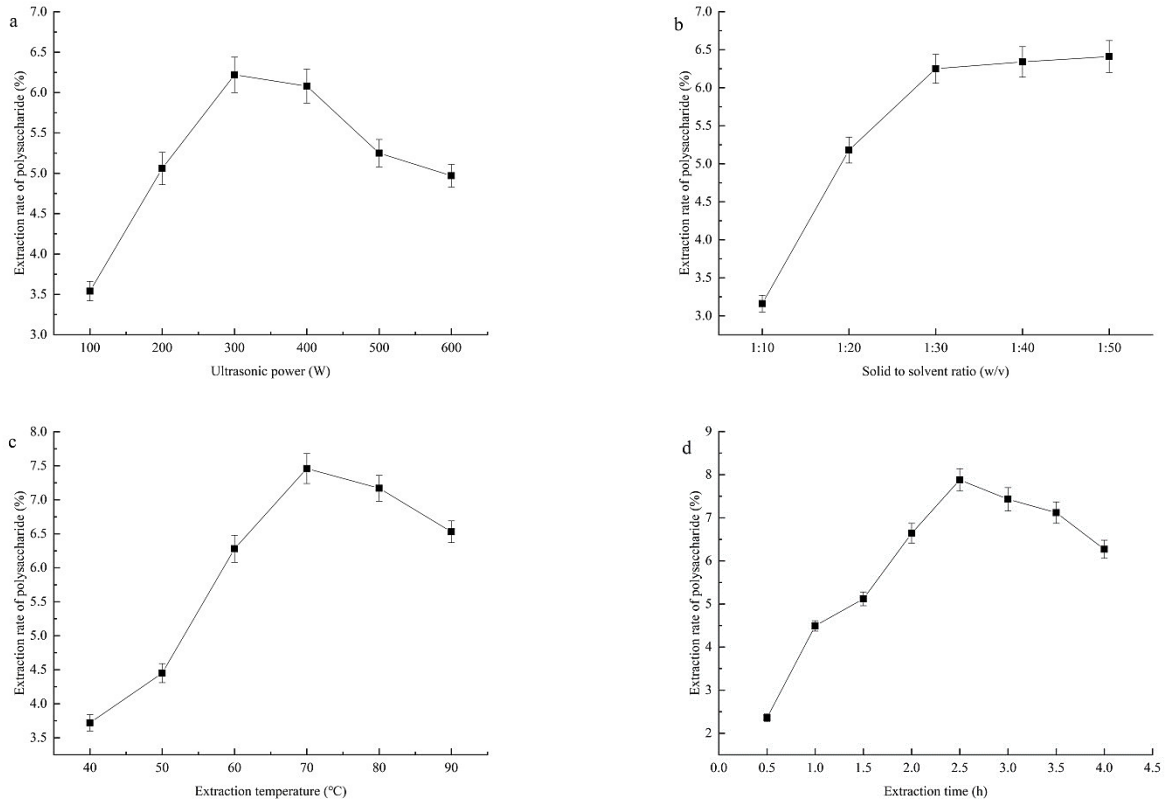

Figure 1. Effects of different factors on extraction rates of polysaccharides; **(a)** ultrasonic power, **(b)** solid to solvent ratio, **(c)** extraction temperature, and **(d)** extraction time.

(Fabregas *et al*., 1999). However, an excessively low solid-to-solvent ratio would increase the raw materials and reagents amount, cause waste, and increase costs, as well as increase the difficulty of the post-treatment process. Therefore, the solid-to-solvent ratio was chosen to be 1:30 (w/v), which could ensure a high extraction rate, and reduce the extraction solution amount.

The extraction rate of polysaccharide increased rapidly, and then decreased gradually with the increase in extraction temperature (Figure 1c). The extraction rate of polysaccharide reached the highest at 7.46% when the temperature was 70°C. This was probably because the solubility of polysaccharide would increase with the extraction temperature rise, and the dissolution amount of polysaccharide would increase, leading to the increase in extraction rate. However, the colour of the extracted polysaccharide was darker when the extraction temperature was too high, which might be because the polysaccharide was easy to degrade at high temperature (Sun *et al*., 2017), and high temperature would increase energy consumption. Therefore, 70°C was chosen as the suitable extraction temperature.

Within 0.5~2.5 h, the extraction rate of polysaccharide gradually increase (Figure 1d). The extraction rate reached the maximum value of 7.88% when the extraction time was 2.5 h. However, the extraction rate gradually decreased after 2.5 h. The longer the extraction time was, the more beneficial it was to polysaccharide dissolution, which resulted in the increase in the extraction rate within a certain period. However, the polysaccharide would be degraded if the extraction time were too long, which would lead to a decrease in the extraction rate. Therefore, the extraction time was chosen to be 2.5 h.

Central Composite Design

Design-expert 8.0 was used to design the four-factor and three-level central combination test. The response value was the extraction rate of polysaccharide. The design scheme and results of the 27 experimental combinations are shown in Table 1.

Model establishment and significance test

The Design-expert software was used to analyse the experimental results, and the fitted equation was as follows (Eq. 6):

$$Y = 8.71 - 0.43X_1 + 0.17X_2 + 0.10X_3 + 0.40X_4 + 0.077X_1X_2 - 0.24X_1X_3 + 0.074X_1X_4 + 0.32X_2X_3 + 6.250E - 004X_2X_4 - 0.028X_3X_4 - 0.66X_1^2 - 0.043X_2^2 - 0.22X_3^2 - 0.018X_4^2 \quad \text{(Eq. 6)}$$

The analysis of variance results of the regression equation by analysis software are shown in Table 2.

Table 2. Analysis of variance (ANOVA) of test results.

Source	Sum of squares	df	Mean square	*F*-value	*p*-value, Prob > *F*	
Model	22.68	14	1.62	11.53	< 0.0001	significant
X_1	4.43	1	4.43	31.52	0.0001	**
X_2	0.66	1	0.66	4.67	0.0516	
X_3	0.25	1	0.25	1.78	0.2069	
X_4	3.78	1	3.78	26.93	0.0002	**
X_1X_2	0.095	1	0.095	0.67	0.4281	
X_1X_3	0.90	1	0.90	6.39	0.0265	*
X_1X_4	0.089	1	0.089	0.63	0.4428	
X_2X_3	1.66	1	1.66	11.80	0.0049	**
X_2X_4	6.250E-006	1	6.250E-006	4.447E-005	0.9948	
X_3X_4	0.013	1	0.013	0.090	0.7692	
X_1^2	9.28	1	9.28	66.02	< 0.0001	**
X_2^2	0.040	1	0.040	0.28	0.6040	
X_3^2	1.03	1	1.03	7.31	0.0192	*
X_4^2	7.089E-003	1	7.089E-003	0.050	0.8261	
Residual	1.69	12	0.14			
Lack of fit	1.21	10	0.12	0.50	0.8121	not significant
Pure error	0.48	2	0.24			

*significant, $p < 0.05$; **very significant, $p < 0.01$.

It was apparent that the model was highly significant ($p < 0.01$). The *p*-values of X_1, X_4, X_1X_3, X_2X_3, X_1^2, and X_3^2 in the model were all less than 0.05, indicating that they were all significant; in other words, they had significant effects on the extraction rate of polysaccharide. The lack of fit was 0.8121, and not significant ($p > 0.05$), which indicated that the model was properly selected.

In addition, the correlation coefficient R^2 of the model was 0.9308, which could explain 93.08% of experimental results, indicating that the model had good correlation. The C.V. of the model was 4.76%, which indicated that the experimental operation was reliable, and could accurately reflect the experimental results. In conclusion, the model had a good fitting degree and small experimental error, which could be used to analyse and predict the extraction rate of polysaccharide.

Among the four factors, the influence of ultrasonic power and extraction time was extremely significant. The order of four factors affecting the extraction rate was as follows: $X_1 > X_4 > X_2 > X_3$.

Response surface analysis

In order to analyse the interaction between various factors and determine the best experimental point, a response surface graph was drawn by Design-expert software to evaluate the interaction of each factor on the extraction effect. The response surface graph could visually show the interaction between various factors and the optimal parameters (Yu *et al*., 2017). The response surface graphs of the interaction between various factors are shown in Figure 2. The response surface slopes in Figures 2b and 2e were both relatively steep, indicating that the interaction between X_1 and X_3, X_2, and X_4 had significant effect on the extraction rate. However, the response surface slopes in Figures 2a, 2c, 2d, and 2f were relatively gentle, indicating that the interaction among other factors had no significant influence on the extraction rate.

Confirmatory experiment

Through further analysis of software, the corresponding values of each factor (X_1, X_2, X_3, and X_4) at the maximum response value (Y) were: X_1 = 279.20 W, X_2 = 1:21.74 (w/v), X_3 = 78.98°C, and X_4 = 3.50 h. Under these conditions, the theoretical extraction rate of polysaccharide was 10.2412%.

The reliability of the results was verified based on the optimal value of each factor determined by the response surface. According to the feasibility of actual operation, the optimal process conditions were modified to ultrasonic power 280 W, solid-to-solvent ratio 1:22 (w/v), extraction temperature 79°C, and extraction time 3.5 h. Under the modified optimal experimental conditions, multiple repeated experiments were performed, and the average extraction rate of polysaccharide was 10.11%, which was very close to the theoretical value, and the relative error was only 1.28%, indicating that the regression model had high accuracy.

Separation and purification of polysaccharide

The separation and purification result of crude polysaccharide by DEAE-52 is shown in Figure 3a. Due to the existence of some charged groups, such as sulphuric acid group and uronic acid in polysaccharide components, the components with single charge were preliminarily separated based on ionic strength. PGLP-1, PGLP-2, PGLP-3, and PGLP-4 were eluted by 0, 0.10, 0.20, and 0.30 mol/L NaCI solution, respectively. Due to the low content of PGLP-1 and PGLP-3, the components PGLP-2 and PGLP-4 were dialysed, vacuum freeze-dried, and further purified.

PGLP-2 and PGLP-4 were further purified by the Sephadex G-100 column. Two components, PGLP-2-1 and PGLP-2-2, were obtained from PGLP-2, but the content of PGLP-2-2 was very low (Figure 3b). Only one component, PGLP-4-1, was eluted from PGLP-4 (Figure 3c). PGLP-2-1 and PGLP-4-1 were dialysed and vacuum freeze-dried to obtain refined polysaccharide.

Characterisation of PGLP-2-1 and PGLP-4-1

In the UV-Vis spectra, the absorption peak at 260 or 280 nm indicated that the samples might contain nucleic acids, proteins, or peptides (Hu *et al*., 2017). The UV-Vis absorption spectra of PGLP-2-1 and PGLP-4-1 are shown in Figure 4a. No obvious absorption peaks of PGLP-2-1 and PGLP-4-1 existed between 200 - 500 nm, and a downward trend was revealed with the wavelength increase, which was consistent with the general features of polysaccharide. In addition, no absorption peaks of PGLP-2-1 and PGLP-4-1 existed at 260 and 280 nm, indicating no proteins, peptides, and nucleic acids in PGLP-2-1 and PGLP-4-1.

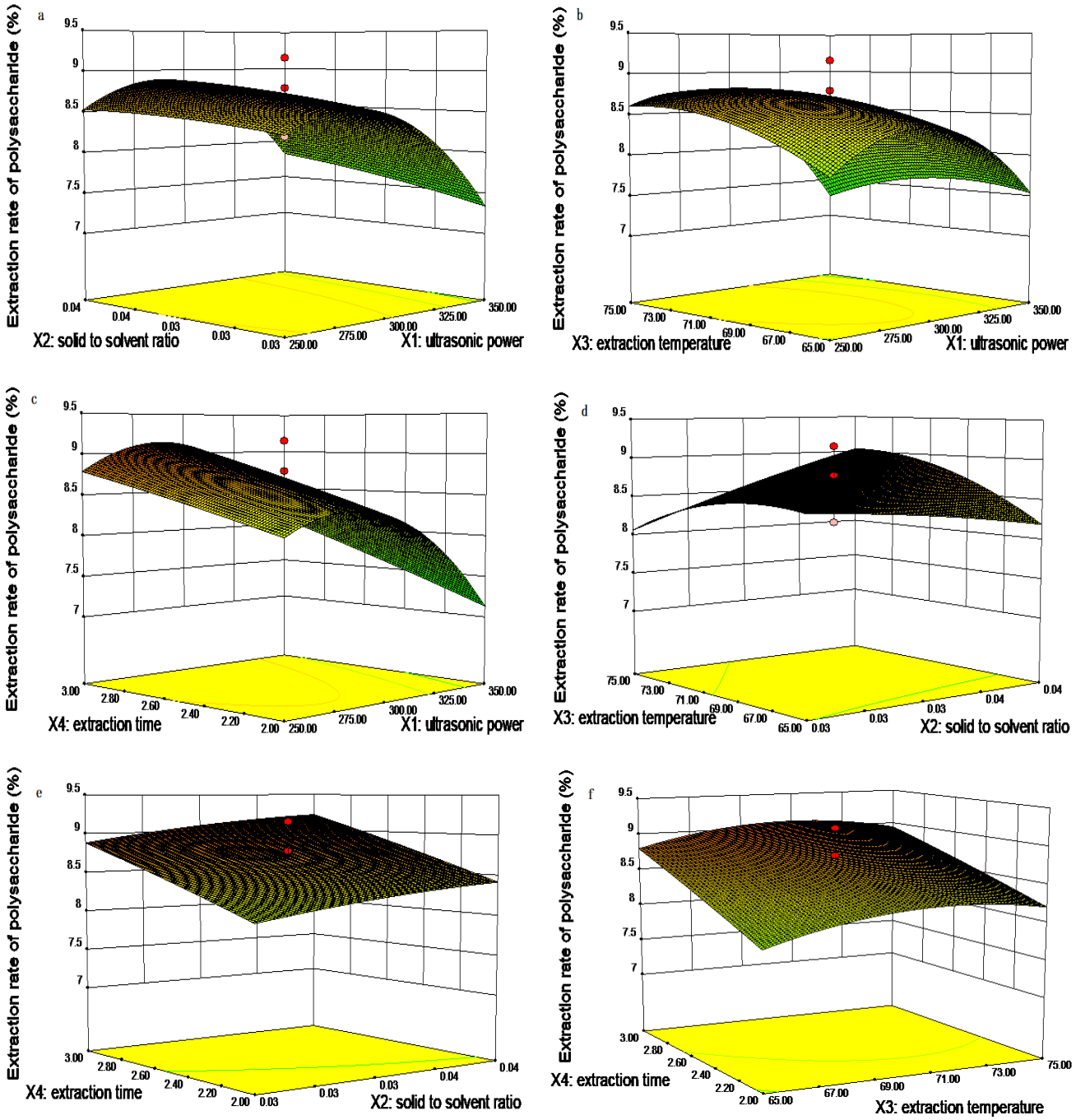

Figure 2. Effects of different factors on extraction rates of polysaccharides.

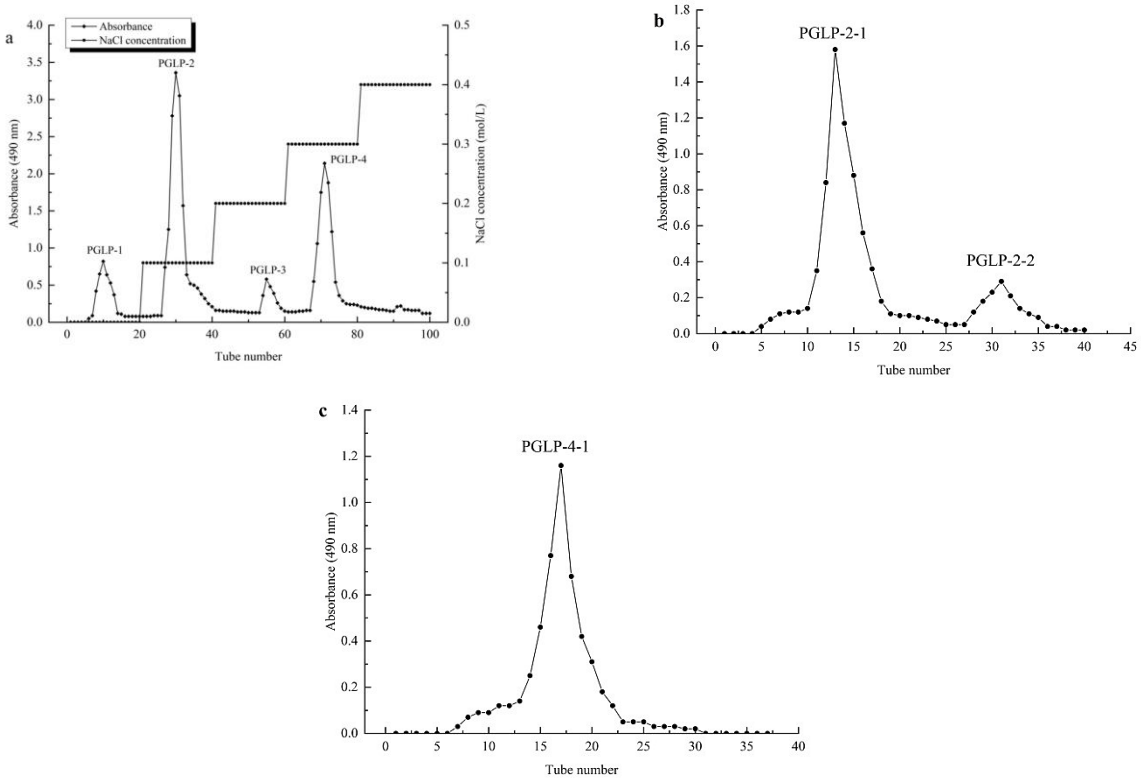

Figure 3. Elution curve of polysaccharides by different chromatographic columns; **(a)** elution curve of DEAE-52 cellulose column; and **(b)** and **(c)** elution curves of Sephadex G-100.

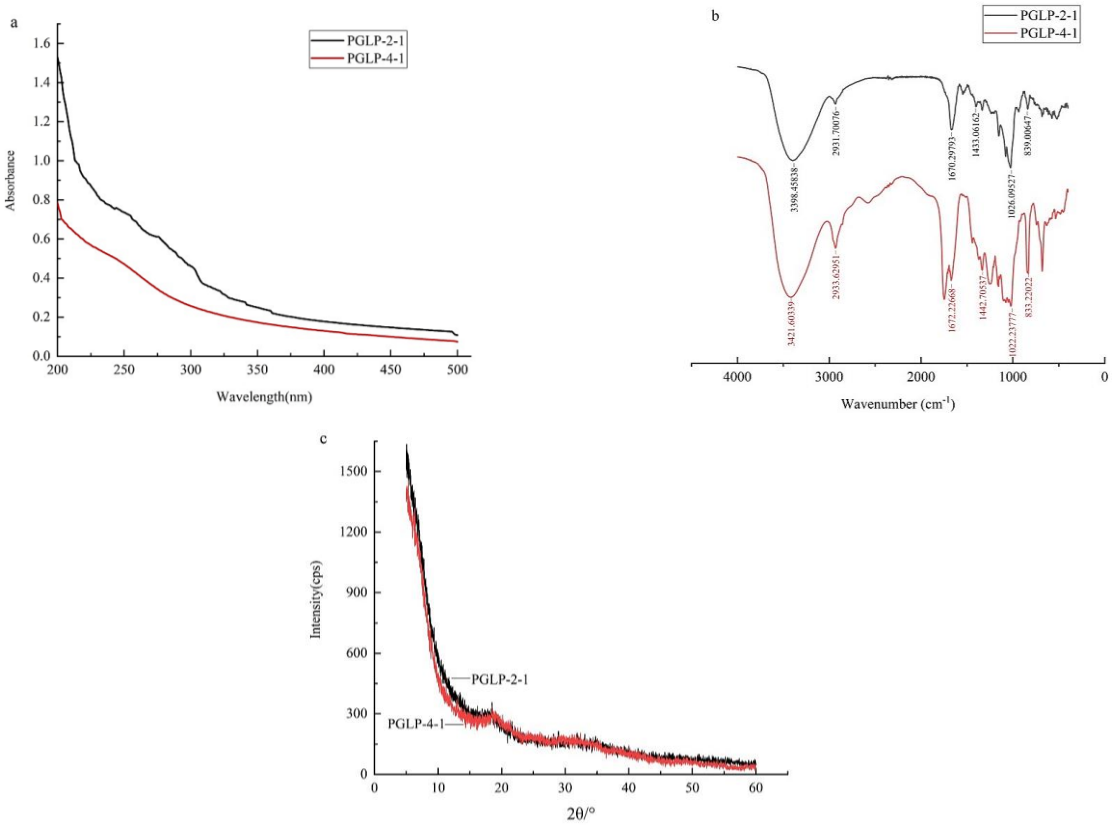

Figure 4. UV-Vis, FT-IR, and XRD of PGLP-2-1 and PGLP-4-1. **(a)** UV-Vis absorption spectrum, **(b)** FT-IR spectrum, and **(c)** XRD profile.

The FT-IR spectrum of PGLP-2-1 and PGLP-4-1 showed vibrational bands which were typical of carbohydrates (Figure 4b). The strong broad absorption peak near 3400 cm^{-1} indicated the presence of inter- and intramolecular hydrogen bonds, which could be attributed to the stretching vibrations of O-H (Ouyang *et al*., 2021). The signal at around 2931 cm^{-1} could be associated with the stretching vibration of the C–H bond in the sugar ring (Romdhanea *et al*., 2017). The bands at around 1670 cm^{-1} were due to the bond stretching vibrations of C=O bonds in the acyl-amino group. The band around 1430 cm^{-1} was assigned to the bending vibration of the C–H bond (Wang *et al*., 2017b). PGLP-2-1 and PGLP-4-1 had a specific band in the 1200 - 1000 cm^{-1} region, which was dominated by ring vibrations overlapped with stretching vibrations of C-OH and C-O-C (Liu *et al*., 2016). The absorption peak near 840 cm^{-1} indicated that PGLP-2-1 and PGLP-4-1 had C-H variable-angle vibrations.

The XRD analysis results of PGLP-2-1 and PGLP-4-1 are shown in Figure 4c. No obvious absorption peaks of PGLP-2-1 and PGLP-4-1 existed in the range of 5° - 60°, indicating the amorphous structure of the samples (Sun *et al*., 2023).

Antibacterial activity of PGLP-2-1 and PGLP-4-1

PGLP-2-1 had the strongest antibacterial activity against *S. aureus*, and the diameter of the inhibition zone could reach 17.46 mm; besides, it also had certain antibacterial activity against *P. aeruginosa*, *S.* Typhimurium, and *B. subtilis*, but the inhibition zone was small, and the antibacterial activity was weak; it had no inhibitory effect against *E. coli* and *P. mulfocida* (Table 3). PGLP-4-1 had weak antibacterial activity against *P. aeruginosa*, *S.* Typhimurium, and *S. aureus*, but no inhibitory effect against *E. coli*, *P. mulfocida*, and *B. subtilis* (Table 3).

In conclusion, PGLP-2-1 had stronger inhibitory effect against Gram-positive bacteria, and weak inhibitory effect against Gram-negative bacteria. PGLP-4-1 had stronger inhibitory effect against Gram-negative bacteria than Gram-positive bacteria.

Table 3. Antibacterial activity of PGLP-2-1 and PGLP-4-1.

Strain		Inhibitory zone diameter (mm)			
		PGLP-2-1	**PGLP-4-1**	**Positive control**	**Blank control**
Gram-positive bacterium	*Staphylococcus aureus*	17.46 ± 0.4	12.52 ± 0.3	18.72 ± 0.5	−
	Bacillus subtilis	12.88 ± 0.3	−	19.56 ± 0.6	−
Gram-negative bacterium	*Escherichia coli*	−	−	23.95 ± 0.7	−
	Pseudomonas aeruginosa	9.58 ± 0.2	12.24 ± 0.2	17.58 ± 0.4	−
	Salmonella Typhimurium	10.24 ± 0.2	11.35 ± 0.2	22.88 ± 0.6	−
	Pastevula mulfocida	−	−	16.52 ± 0.4	−

−: no antibacterial activity.

Many natural polysaccharides from plants have antibacterial activity against both Gram-positive and Gram-negative bacteria. Fu (2014) studied the antibacterial activity of polysaccharides from *P. grandiflorus in vitro*, and found that polysaccharides had good antibacterial activity against *S. aureus*, *E. coli*, *S.* Typhimurium, and *B. subtilis*. Natural polysaccharides mainly achieve antibacterial effect by destroying the cell wall and membranes of bacteria, regulating enzyme activity and ion levels in bacteria, regulating energy metabolism, influencing genes, and their antibacterial activities are closely related to the structure-activity relationship of polysaccharide, such as molecular size, polymerisation degree, and constituents.

Antioxidant activity of PGLP-2-1 and PGLP-4-1

The scavenging ability of PGLP-2-1 and PGLP-4-1 to superoxide anion radical increased with the increase in sample concentration, and showed a good dose-effect relationship (Figure 5a). When sample concentration was 1.2 mg/mL, the clearance rate of PGLP-2-1 and PGLP-4-1 to superoxide anion radical reached 86.68 and 75.04%, respectively. Both PGLP-2-1 and PGLP-4-1 had lower scavenging capacity to superoxide anion radical than Vc, while PGLP-2-1 had stronger scavenging ability than PGLP-4-1. The results showed that PGLP-2-1 and PGLP-4-1 had strong scavenging ability to superoxide anion radical.

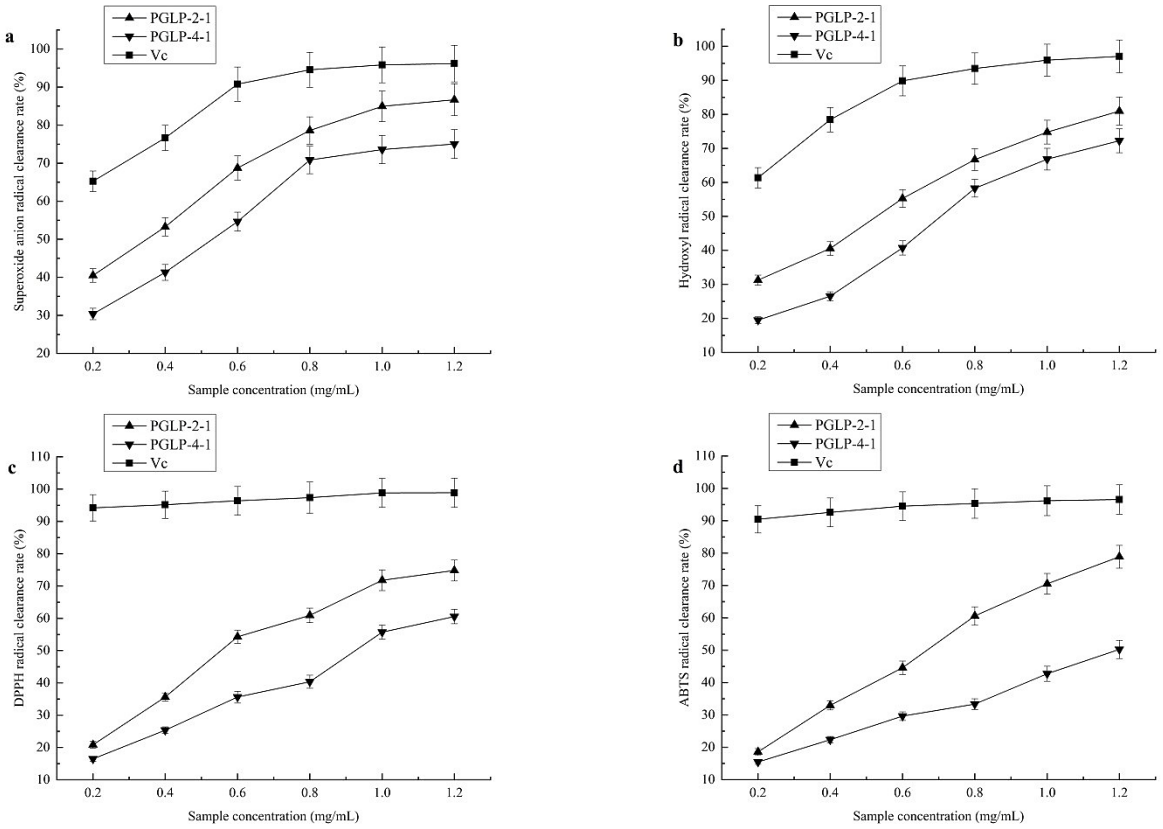

Figure 5. Scavenging ability of polysaccharide; **(a)** scavenging ability to superoxide anion radical, **(b)** scavenging ability to hydroxyl radical, **(c)** scavenging ability to DPPH radical, and **(d)** scavenging ability to ABTS radical.

Hydroxyl radical can cause serious damage to macromolecules such as proteins and nucleic acids, so the scavenging effect of test substances on hydroxyl radical is also an important indicator to measure its antioxidant capacity (Guo *et al*., 2019). The scavenging ability of PGLP-2-1 and PGLP-4-1 to hydroxyl radical increased with the increase in sample concentration (Figure 5b). When sample concentration was 1.2 mg/mL, the clearance rate of PGLP-2-1 and PGLP-4-1 to hydroxyl radical reached 80.96 and 72.23%, respectively. PGLP-2-1 and PGLP-4-1 had lower scavenging capacity to hydroxyl radical than Vc, while PGLP-2-1 had stronger scavenging ability than PGLP-4-1. The results showed that PGLP-2-1 and PGLP-4-1 had strong scavenging ability to hydroxyl radical.

The scavenging ability of PGLP-2-1 and PGLP-4-1 on DPPH free radical increased with the increase in sample concentration in a concentration-dependent manner (Figure 5c). When the sample concentration was 1.2 mg/mL, the scavenging rates of PGLP-2-1 and PGLP-4-1 on DPPH free radical were the highest, reaching 74.82 and 60.56%, respectively. The scavenging ability of PGLP-2-1 and PGLP-4-1 on DPPH free radical was lower than Vc, while PGLP-2-1 had stronger scavenging ability on DPPH free radical than PGLP-4-1. The results showed that PGLP-2-1 and PGLP-4-1 had strong scavenging ability on DPPH free radical.

The scavenging ability of PGLP-2-1 and PGLP-4-1 on ABTS free radical increased with the increase in sample concentration in a concentration-dependent manner (Figure 5d). When the sample concentration was 1.2 mg/mL, the scavenging rates of PGLP-2-1 and PGLP-4-1 on ABTS free radical were the highest, reaching 78.88 and 50.25%, respectively. The scavenging ability of PGLP-2-1 and PGLP-4-1 on ABTS free radical was lower than Vc, while PGLP-2-1 had stronger scavenging ability on ABTS free radical than PGLP-4-1. The results showed that PGLP-2-1 and PGLP-4-1 had strong scavenging ability on ABTS free radical.

The results showed that PGLP-2-1 and PGLP-4-1 had strong antioxidant activity, and PGLP-2-1 was higher than PGLP-4-1.

Biological oxidation can provide energy for human activities, but excessive free radicals generated by oxidation may cause damage to the

normal cells and tissues of the human body, leading to a variety of diseases, such as cancers, arteriosclerosis, diabetes, cataracts, cardiovascular diseases, Alzheimer's disease, and arthritis (Mu *et al*., 2021). Zou *et al*. (2021) isolated a pectin polysaccharide PGP-I-I from the root of *P. grandiflorus*, and found that PGP-I-I could restore the antioxidant defence of intestinal cells by promoting the expression of cellular antioxidant genes, and play a protective role against oxidative damage under the condition of hydrogen peroxide treatment. Plant polysaccharides could regulate the expression of related inflammatory factor genes *in vivo* or cells by regulating the inflammatory response signalling (NF-κB) pathway, so as to alleviate the damage caused by oxidative stress. In addition, the MAPK/Nrf2 signalling pathway could promote the secretion and increase the activity of antioxidant enzymes under the regulation of plant polysaccharides, which could reduce the level of free radicals in the body, thus enhancing the resistance of the body to oxidative stress.

The monosaccharide composition, relative molecular weight, glycosidic bond type, degree of substitution, and spatial conformation of plant polysaccharides can affect their biological activities, and the primary and higher structure of polysaccharides can directly or indirectly affect their active functions (Yin *et al*., 2015). Therefore, the antibacterial and antioxidant mechanisms of polysaccharide from *P. grandiflorus* leaves need to be further studied.

Conclusion

In the present work, the extraction process of PGLP was optimised by the response surface method. Then, the antibacterial and antioxidant activities of polysaccharide were studied after isolation and purification. After optimisation, the optimal solution for polysaccharide extraction was as follows: ultrasonic power, 280 W; solid-to-solvent ratio, 1:22 (w/v); extraction temperature, 79°C; and extraction time, 3.5 h. Under these conditions, the extraction rate was 10.11%, which was consistent with the theoretical value. The crude polysaccharide from *Platycodon grandiflorus* leaves was isolated and purified, and two components PGLP-2-1 and PGLP-4-1 were obtained. UV-Vis, FT-IR, and XRD analyses showed that PGLP-2-1 and PGLP-4-1 had typical characteristics of polysaccharides.

Bacteriostatic experiment results showed that PGLP-2-1 had the strongest antibacterial activity against *S. aureus*, and weaker antibacterial activity against *P. aeruginosa*, *S.* Typhimurium, and *B. subtilis*; PGLP-4-1 had weak antibacterial activity against *P. aeruginosa*, *S.* Typhimurium, and *S. aureus*. Antioxidant activity experiment results showed that PGLP-2-1 and PGLP-4-1 had strong antioxidant activity, and the antioxidant activity of PGLP-2-1 was higher than PGLP-4-1. PGLP-2-1 had strong scavenging ability on superoxide anion, hydroxyl, DPPH, and ABTS free radicals, and the highest scavenging rate reached 86.68, 80.96, 74.82, and 78.88%, respectively. PGLP-4-1 also had strong scavenging ability on superoxide anion, hydroxyl, DPPH, and ABTS free radicals, and the highest scavenging rate reached 75.04, 72.23, 60.56, and 50.25%, respectively. Therefore, the present work provided a theory gist for the comprehensive utilisation of *P. grandifloras* leaves, and a certain theoretical basis for screening natural antibacterial and antioxidant active substances.

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