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Optimization, characterization, hypoglycemic and antioxidant activity studies of ultrasonic-assisted deep eutectic solvent extraction of polysaccharides from *Perilla frutescens*

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ABSTRACT

Objective: In order to further develop and utilise *Perilla frutescens* polysaccharides, the aim of this study was to optimize process conditions for the extraction of *Perilla frutescens* polysaccharides with deep eutectic solvents (DES), to investigate their biological activities and to analyse their monosaccharide composition.
Methods: Perilla frutescens polysaccharides were extracted by DES and ultrasonic extraction, and the optimal extraction process was obtained by one-way test and response surface method. The extract was collected and concentrated, alcohol precipitated, deproteinized, dialyzed, and evaporated to make a solution, which was purified by DEAE and Sephadex G-75 columns to obtain the arginal polysaccharide PFP-1.
Results: The polysaccharide was extracted from *Perilla frutescens* by ultrasonication with the aid of a new type of DES, and the optimal conditions for extraction were determined: the optimal DES system for extracting the polysaccharide of *Perilla frutescens* and the molar ratio was choline chloride: oxalic acid = 2:1; ultrasonication temperature, power, and time were 70 °C, 280 W, and 45 min, respectively; and the liquid-solid ratio was 21 mL/g, and the extraction rate of polysaccharide was 2.44%. The molecular weight (*Mw*) of PFP-1 is approximately 11600 Da. Hydrolysis and derivatization of the refined polysaccharide PFP-1 followed by analytical experiments determined that the polysaccharide monosaccharides consisted of rhamnose, glucuronic acid, and glucose, with the molar ratios of 1.45: 2.82: 1.41. The antioxidant activity and hypoglycemic activity of the polysaccharide were determined to be good by free radical scavenging and enzyme inhibition experiments, and the IC_{50} of *Perilla frutescens* polysaccharide against DPPH• was 0.117 mg/mL and that against α-amylase was 0.002 mg/mL.
Conclusion: This study can provide an experimental basis for the establishment of an industrialized production process of polysaccharides and the study of their biological activities.

1. Introduction

Perilla frutescens (L.) Britt. (*P. frutescens*) holds significant value as both a medicinal plant, utilized for its stems, leaves, and seeds, and a nutritious food source.[1] Particularly rich in essential micronutrients (e.g., iron, calcium), amino acids, vitamins, and other compounds vital for human health, *P. frutescens* leaves contribute to benefits like atherosclerosis prevention and enhanced metabolism. These leaves harbor diverse bioactive constituents, including flavonoids, phenolic acids, volatile oils, and polysaccharides. Among these components, *P. frutescens* polysaccharides (PFPs) have emerged as a primary focus due to their prominent biological activities.[2,3] Specifically, numerous studies report that PFPs possess significant antioxidant, hypoglycemic, and hypolipidemic effects.[1]

The efficient extraction of such valuable polysaccharides is crucial for their study and application. Traditional extraction methods often have limitations.[4] Therefore, the exploration of advanced, green solvents like deep eutectic solvents (DES) is highly relevant. DES, formed by hydrogen bonding between a Hydrogen Bond Acceptor (HBA) and a Hydrogen Bond Donor (HBD), represent a promising class of environmentally friendly solvents.[5] These solvents are broadly categorized into four types: Type I (quaternary ammonium salt + metal chloride), Type II

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(quaternary ammonium salt + metal chloride hydrate), Type III (quaternary ammonium salt + HBD), and Type IV (metal chloride + HBD). Compared to conventional organic solvents, DESs offer compelling advantages such as low toxicity, biodegradability, and cost-effective preparation (typically via simple heating/stirring),[6] making them increasingly adopted in fields like organic synthesis and bioactive compound extraction.

The choice of extraction technique further influences polysaccharide yield and quality. Common methods include maceration (involving solvent penetration and diffusion at ambient or elevated temperatures), which is simple but often inefficient.[7,8] In contrast, ultrasound-assisted extraction (UAE) employs acoustic cavitation to disrupt plant cell walls, facilitating the release of intracellular components like polysaccharides.[9] UAE offers distinct benefits, including reduced solvent consumption, shorter processing times, and potentially higher extraction yields.[10] Consequently, combining DESs with UAE presents a synergistic approach for efficient polysaccharide recovery.

The interest in plant polysaccharides like PFPs stems directly from their multifaceted biological activities. PFPs specifically demonstrate pronounced antioxidant capacity. Standardized *in vitro* assays for quantifying natural product antioxidant activity include measuring scavenging rates against ABTS$\bullet^+$ and DPPH$\bullet$ radicals.[1] For example, 2000 μg/mL of a *perilla* seed meal polysaccharides (PSMP) showed a scavenging rate on DPPH$\bullet$ radical of 75%.[11] Plant polysaccharides also possess demonstrated hypoglycemic effects, assessable via inhibition assays targeting α-glucosidase and α-amylase. However, research on both antioxidant and hypoglycemic activities in *perilla* leaf polysaccharides is currently absent.

Given the substantial biological potential and application value of PFPs, coupled with the promise of DES-based extraction, this study aims to: (1) Optimize the extraction of PFPs using DESs combined with UAE, employing single-factor experiments and Response Surface Methodology (RSM)[12] to identify the optimal process conditions; (2) Evaluate the *in vitro* antioxidant activity of the extracted PFPs using ABTS$\bullet^+$ and DPPH$\bullet$ radical scavenging assays; (3) Assess their hypoglycemic potential via α-glucosidase and α-amylase inhibition assays; (4) Determine the monosaccharide composition of the purified polysaccharides. This comprehensive investigation seeks to establish a robust foundation for improving the industrial-scale production of PFPs and advancing their development for functional foods and healthcare applications.

2. Materials and methods

2.1. Materials and reagents

P. frutescens was obtained from Guilin, Guangxi. The material was authenticated by Prof. B. Liu, Guangdong Pharmaceutical University, Guangzhou, China. Voucher specimens were kept at the School of Pharmacy, Guangdong Pharmaceutical University, Guangdong, China. Sephadex G-75 and DEAE-SephRrosa were purchased from Lanxiao Science and Technology New Material Co., Ltd. (Xi'an, China). Acarbose and p-nitrophenyl-α-D-glucopyranoside (PNPG) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). α-Glucosidase, ABTS (2,2-diazobis-3-ethylbenzothiazoline-6-sulfonic acid), D-(+)-anhydrous glucose, rhamnose, D-(+)-galacturonic acid, D-galactose, and D-glucuronic acid were purchased from Haruno Biotechnology Ltd. (Shanghai, China). (Shanghai, China). α-Amylase, DPPH (1,1-diphenyl-3-nitrophenylhydrazine), and PMP (1-phenyl-3-methyl-5-pyrazolone) were purchased from McLean Biochemistry & Technology Co. (Shanghai, China). DNS reagent was purchased from Feijing Biotechnology Co. (Shanghai, China). DNS reagent was purchased from Feijing Biotechnology Co. L-arabinose, D-mannose, D-(+)-xylose, and L-(−)-fucose were supplied by Desi Biotech Co. All other chemicals and solvents were of analytical grade.

2.2. Drawing of standard curves

Anhydrous glucose (4 mg) was accurately weighed and dissolved to prepare a 0.4 mg/mL standard solution.[13] Aliquots (10, 20, 30, 40, and 50 μL) of this solution were pipetted into EP tubes and brought to a final volume of 100 μL with distilled water. Color development was performed using the phenol-sulfuric acid method (reagent ratio: phenol: sulfuric acid:sample volume = 1:1:5). After sequential addition of phenol solution and concentrated sulfuric acid, the mixtures were vortex-mixed and incubated at room temperature for 15 min. Absorbance of 200 μL reaction mixture was measured at 492 nm. All samples were analyzed in triplicate, and the standard curve was constructed from mean absorbance values.

2.3. Extraction rate determination

The extraction protocol was adapted from Ref. 14. Powdered *P. frutescens* (0.5 g) was mixed with a low eutectic solvent (DES)-water system at specified liquid-solid ratios and water contents. Following thorough mixing, polysaccharides were extracted under ultrasonication at optimized conditions. The extract was centrifuged, and the supernatant was collected for polysaccharide quantification.

The combined supernatants were concentrated and subjected to ethanol precipitation (adjusted to 80% final ethanol concentration using an alcohol meter) for 12 h at 4 °C. After centrifugation (4000×g, 5 min), the precipitate was collected, redissolved in distilled water, and deproteinized via the Sevag method (polysaccharide solution: CH_2Cl_2: *n*-butanol = 25:4:1 v/v). The mixture was agitated for 30 min, centrifuged, and the aqueous phase was concentrated, dialyzed (Mw cut-off: 1000 Da) against running water (48 h), and lyophilized.

Content was determined by the phenol-sulfuric acid method. Samples (20 μL) were reacted with phenol solution and concentrated H_2SO_4 (sample:phenol:H_2SO_4 1:1:5 v/v) for 15 min. After dilution to 200 μL with distilled water in 96-well plates, absorbance was measured at 492 nm using reagent blanks. Extraction yield was calculated as:

$$\text{Polysaccharide extraction rate} = C \times V \times n/M \times 100\% \tag{1}$$

Where C = glucose-equivalent concentration (mg/mL), V = total extraction volume (mL), n = dilution factor, and M = sample mass (g).

2.4. Screening of deep eutectic solvents

According to method[15] with slight modification, 7 groups of different DES were prepared. A certain amount of DES preparation was weighed according to the molar ratio and melted in a water bath at 80 °C while stirring until all the solids melted into a homogeneous and clear liquid. Seven DES were used as solvents for extraction, including DES-1 (choline chloride-ethylene glycol, molar ratio 1:2), DES-2 (choline chloride-malonic acid, molar ratio 1:1), DES-3 (choline chloride-propanetriol, molar ratio 1:2), DES-4 (choline chloride-1, 4-butanediol, molar ratio 1:4), DES-5 (choline chloride-oxalate, molar ratio 2:1), DES-6 (choline chloride-urea, molar ratio 1:2), and DES-7 (choline chloride-citric acid, molar ratio 2:1).

The extraction rate of *P. frutescens* polysaccharides was used as an indicator, and the group with the highest polysaccharide extraction rate was selected as the solvent for subsequent experiments.

2.5. Single-factor experiment

Precisely weighed *P. frutescens* powder underwent polysaccharide extraction using DES. Extraction conditions were systematically varied: DES molar ratio (HBA:HBD; 1:1, 1.5:1, 2:1, 2.5:1, 3:1), water content (10%, 30%, 50%, 70%, 90%), extraction temperature (20, 35, 50, 65, 80 °C), liquid-solid ratio (10, 15, 20, 25, 30 mL/g), ultrasonic power (200, 240, 280, 320, 360 W), and ultrasonic time (15, 30, 45, 60, 75 min).

Water content is expressed as weight percentage (wt%). DES were mixed at 80 °C for 2 h until homogeneous. Deionized water (10%–90% wt relative to total DES) was then added at room temperature with stirring.

2.6. Response surface experimental design

Based on the results of the one-way experiments, 4 factors and 3 levels were selected as response values for the polysaccharide extraction rate of *P. frutescens*, and the Box-Behnken design in Design-Expert 12.0 was used for the experimental design of response surfaces, obtaining 29 experimental condition runs. The experiments were carried out based on the design results and the corresponding polysaccharide extraction rates were obtained. The optimum extraction process was analyzed, validated, and compared with ultrasonic water extraction and water immersion methods.

2.7. Purification

2.7.1. DEAE-sepharose purification

A slight modification was made according to method.[16] After concentrating the crude extract, 200 mg of crude polysaccharide was dissolved in 10 mL of distilled water and centrifuged (4000 rpm, 5 min), the supernatant was retained to obtain the sample solution. 10 mL of the sample solution was collected in 10 mL tubes on a DEAE-SephaRosa Fast Flow column with gradient elution at a flow rate of 2 mL/min 50 Tubes were first eluted with distilled water and then 80 tubes were eluted with a gradient elution of 1.0 M NaCl. The absorbance values were measured at 492 nm to obtain an elution curve through which the peak components were collected, dialyzed (Mw cut-off: 1000 Da) in running water for 24 h, concentrated, and dried to obtain the sample.

2.7.2. Sephadex G-75 purification

Distilled water was added to the sample to make a 10 mg/mL sample solution. 4 mL of sample solution was loaded onto a Sephadex G-75 column. 4 mL of sample solution was loaded onto a Sephadex G-75 column and eluted with water at a flow rate of 0.8 mL/min 10 mL of eluate was collected from each tube, and a total of 50 tubes were collected. The color was developed by the phenol-concentrated sulfuric acid method, the absorbance was measured at 492 nm, the elution curve was plotted and the peak components were collected.

The purity was calculated by the following formula.

$$\text{Purity } (\%) = c \times V / m \times 100\% \tag{2}$$

Where c (mg/mL) is the concentration of PFP, V (mL) is the volume of the sample solution, and m (mg) is the mass of the sample.

2.8. Homogeneity and M_w determination

The dextran standard with molecular weights of 668000, 410000, 273000, 148000, 48600, 23800, 11600, and 5200 Da was dissolved in the mobile phase. respectively, and then prepared into a solution with a concentration of 2 mg/mL. After passing through the membrane, the dextran standard was analyzed by a high-performance gel permeation chromatography (HPGPC) system. The calibration curve equation was obtained from the logarithm of M_w (log M_w) and elution volume (V) of the dextran standard. Dissolved the polysaccharides from *P. frutescens* leaf in the mobile phase to prepare a solution with a concentration of 2 mg/mL, passed it through a membrane, and analyzed it using HPGPC. By using the calibration curve equation obtained above, the molecular weight of each *P. frutescens* leaves polysaccharide can be determined. HPGPC conditions: Analytical instrument: High-performance liquid chromatography Waters 1525; Detector: Refractive index detector Waters 2414; Column temperature: 35 °C; Gel column: Ultrahydrogel 1000 (7.8 × 300 mm), Ultrahydrogel 500 (7.8 × 300 mm); Mobile phase: 0.02 M KH_2PO_4 solution; Flow rate: 0.8 mL/min; Injection volume: 20 μL.

2.9. Analysis of monosaccharide components

2.9.1. Analytical conditions

By using HPLC (Agilent 1260), Kromasil 100-5-C_{18} column (5 μm, 4.6 × 250 mm), column temperature (25 °C), and ultraviolet detector (250 nm), 10 μL of the sample was injected. Under the flow rate of 1 mL/min, 0.05 M phosphate buffer salt (pH 6.7) -acetonitrile served as the mobile phase at the proportion of 85:15 (v/v).

2.9.2. Hydrolysis and pre-column derivatization of PFP-1 by PMP

The method for obtaining the derivatives was based on the literature with slight modifications.[17] PFP-1 was completely hydrolyzed with trifluoroacetic acid (TFA) in an oil bath at 120 °C for 4 h. The hydrolysate was then transferred to a 50 mL round-bottomed flask and concentrated to dryness at 65 °C under reduced pressure. Then 6 mL of methanol was added and dried again, and the process was repeated three times to remove the TFA. The spin-dried solid was dissolved in 1 mL of deionized water to form a hydrolysate. In a 5 mL centrifuge tube, 100 μL of hydrolysis solution, 0.5 M PMP-methanol solution, and 0.3 M NaOH solution were added. After mixing, the reaction was carried out in a water bath at 70 °C for 30 min. After cooling, 105 μL of hydrochloric acid solution (0.3 M) was added and then diluted with 400 μL of deionized water. The chloroform layer was discarded and repeated twice to remove excess PMP, and finally, the aqueous layer was removed and filtered through a 0.45 μm aqueous system microporous membrane to obtain a mixed monosaccharide sample solution.

2.9.3. Precolumn derivatization of PMP of mixed monosaccharide control products

The experiment was carried out according to the method.[18] The proper amount of glucose, mannose, arabinose, xylose, fucose, glucuronic acid, rhamnose, and galactose was precisely weighed to prepare a standard solution of 2 mM. 50 μL each of the monosaccharide reference solutions was mixed in a 50 mL centrifuge tube. Then 450 μL of 0.3 M NaOH solution and 0.5 M PMP-methanol solution were added, mixed, and reacted in a water bath at 70 °C for 30 min. After cooling, the mixture was neutralized with 460 μL of hydrochloric acid solution (0.3 M). 2 mL of chloroform was added for extraction, and the chloroform layer was discarded. The above operation was repeated twice to reduce PMP residue, and finally, the water layer was taken and filtered through a 0.45 μm water system microporous filter membrane, through which a sample for analysis was made.

2.10. Bioactivity study

2.10.1. α-Glucosidase inhibition test

The assay was performed according to the protocol reported with slight modifications.[19] Different concentrations of sample solution and acarbose solution (3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25 μg/mL, 40 μL) were mixed with α-glucosidase (1 U/mL, 40 μL) in a 96-well plate, and the mixture was incubated for 10 min at 37 °C, and PNPG (3 mM, 20 μL) was added to the mixture and continued to incubate at 37 °C for 30 min, and finally, the reaction was terminated by adding 100 μL Na_2CO_3 solution (1 M). The absorbance values were measured at 405 nm with acarbose as a positive control. Each concentration was measured three times in parallel and the average value was taken. The inhibition rate was calculated by the following formula:

$$\text{Inhibition rate } (\%) = [1\text{-}(A_s\text{-}A_b) / A_0] \times 100\% \tag{3}$$

Where A_0 = α-glucosidase solution + PNPG, A_S = sample + α-glucosidase + PNPG, and A_b = sample + PNPG.

2.10.2. α-Amylase inhibition test

The assay method was slightly modified according to the protocol.[20] Different concentrations of sample solution and acarbose solution

(3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25 μg/mL, 40 μL) were mixed with α-amylase (0.5 mg/mL, 40 μL) in EP tubes, and then incubated at 37 °C for 10 min. 20 μL of 1% starch solution was added to the mixture and the reaction was terminated by adding 100 μL of DNS reagent and incubated at 100 °C for 10 min, and then the mixture was diluted with 1 mL of distilled water. The absorbance was measured at 540 nm with acarbose as a positive control and the inhibition rate was calculated according to the following equation:

$$\text{Inhibition rate } (\%) = [1\text{-}(A_s\text{-}A_b) / A_0] \times 100\% \tag{4}$$

In the formula, A_0 is the absorbance of α-amylase solution with starch solution; A_S is the absorbance of sample solution with α-amylase solution and starch solution; A_b is the absorbance of sample solution with starch solution.

2.10.3. DPPH• radicals scavenging test

A series of sample solutions and vitamin C (VitC) reference solutions (3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25 μg/mL) at different concentrations were prepared according to Scheme[21] with minor modifications to the assay method. 20 μL of each concentration of sample solution or control solution was mixed with 180 μL of DPPH• radical solution in a 96-well plate and shaken on a shaker for 30 min in the dark. Absorbance values were measured at 517 nm. Each concentration was measured three times in parallel, and the average value was taken to calculate the clearance rate, which was calculated as follows.

$$\text{Scavenging rate } (\%) = [1 - (A_1 - A_2) / A_0] \times 100\% \tag{5}$$

In the formula, A_1 is the absorbance of the sample solution with DPPH• radical solution; A_2 is the absorbance of the sample solution with absolute ethanol; A_0 is the absorbance of water with DPPH• radical solution.

2.10.4. ABTS•$^+$ radical scavenging test

A series of sample solutions and VitC reference solutions with different concentrations (3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25 μg/mL) were prepared according to the protocol[22] with slight modifications to the assay method. 40 μL of each concentration of sample solution or control solution was mixed with 160 μL of ABTS•$^+$ working solution (mixed with an equal volume of 7.4 mM ABTS•$^+$ solution and 2.6 mM potassium peroxydisulfate, and diluted with absolute ethanol to A (734 nm) = 0.7 ± 0.02) in a 96-well plate, and the absorbance value was measured at the wavelength of 734 nm by shaking on a shaker in the dark for 30 min. The absorbance value at 734 nm was measured.

Three parallel determinations were made and the ABTS•$^+$ radical scavenging rate was calculated according to the following formula:

$$\text{Scavenging rate } (\%) = [1\text{-}(A_1\text{-}A_2) / A_0] \times 100\% \tag{6}$$

In the formula, A_1 is the absorbance of the sample solution with ABTS•$^+$ working solution; A_2 is the absorbance of the sample solution with absolute ethanol; A_0 is the absorbance of absolute ethanol with ABTS•$^+$ working solution.

2.11. Statistical analysis

All data are presented as mean ± standard deviation (mean ± SD) from at least three independent experiments and were analyzed using GraphPad Prism 8 software (GraphPad Software, Inc.). Statistical comparisons among multiple groups were performed by one-way analysis of variance (ANOVA) followed by Dunnett's test, with $P < 0.05$ considered statistically significant.

3. Results

3.1. Results of standard curves

The standard curve was obtained using the glucose concentration as the horizontal coordinate and the absorbance value as the vertical coordinate, and the regression equation of the curve was A = 3.6643C - 0.0111, $R^2 = 0.9991$, indicating a good fit.

3.2. Results and analysis of DES screening

According to Fig. 1, it can be seen that the results of the extraction rate of *P. frutescens* polysaccharides using different DES varied significantly, and by comparison, it was found that the highest extraction rate was achieved when using DES-5 (Choline Chloride-Oxalic Acid) as the extraction solvent, and therefore this group was selected as the extraction solvent for the subsequent experiments.

3.3. Results of single factor experiment

3.3.1. Influence of molar ratio

The extraction was conducted using DES-5 at various molar ratios (1:1, 1.5:1, 2:1, 2.5:1, and 3:1) while maintaining constant parameters: a liquid-solid ratio of 20 mL/g, ultrasonic power of 280 W, duration of 30 min, and temperature of 65 °C. As depicted in Fig. 2a, the highest polysaccharide extraction yield was observed at a choline chloride to oxalic acid molar ratio of 1.5:1. Beyond this ratio, the yield gradually decreased with further increases in the proportion of choline chloride.

3.3.2. Influence of water content

Under standardized extraction conditions (liquid-solid ratio of 20 mL/g, ultrasonic power of 280 W, duration of 30 min, and temperature of 65 °C), the hydro-modulation effects on polysaccharide extraction efficiency were evaluated across aqueous concentrations ranging from 10% to 90%. As shown in Fig. 2b, the highest polysaccharide yield was achieved at 30% aqueous concentration, beyond which a progressive decline in yield was observed with further increases in water content.

3.3.3. Influence of ultrasonic power

The effect of ultrasonic power on polysaccharide extraction was investigated within a range of 200–360 W, while other conditions remained constant. As illustrated in Fig. 2c, the extraction yield increased with rising ultrasonic power and reached a maximum at 280 W. Beyond this point, a further increase in power resulted in a gradual decrease in the extraction rate.

3.3.4. Influence of liquid-solid ratio

A range of liquid-solid ratios from 10:1 to 30:1 mL/g was evaluated

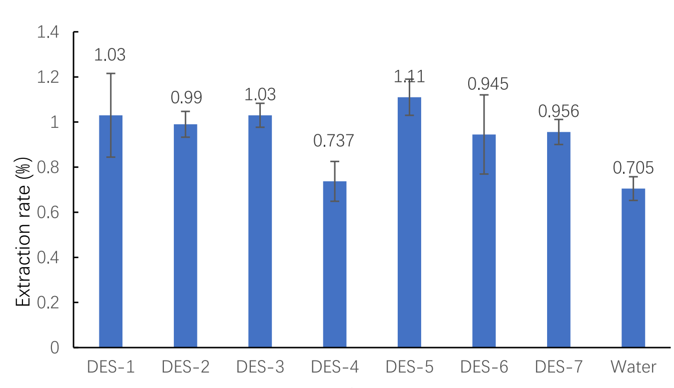

Fig. 1. The extraction rate of *Perilla frutescens* polysaccharides (PFPs) with different DES.

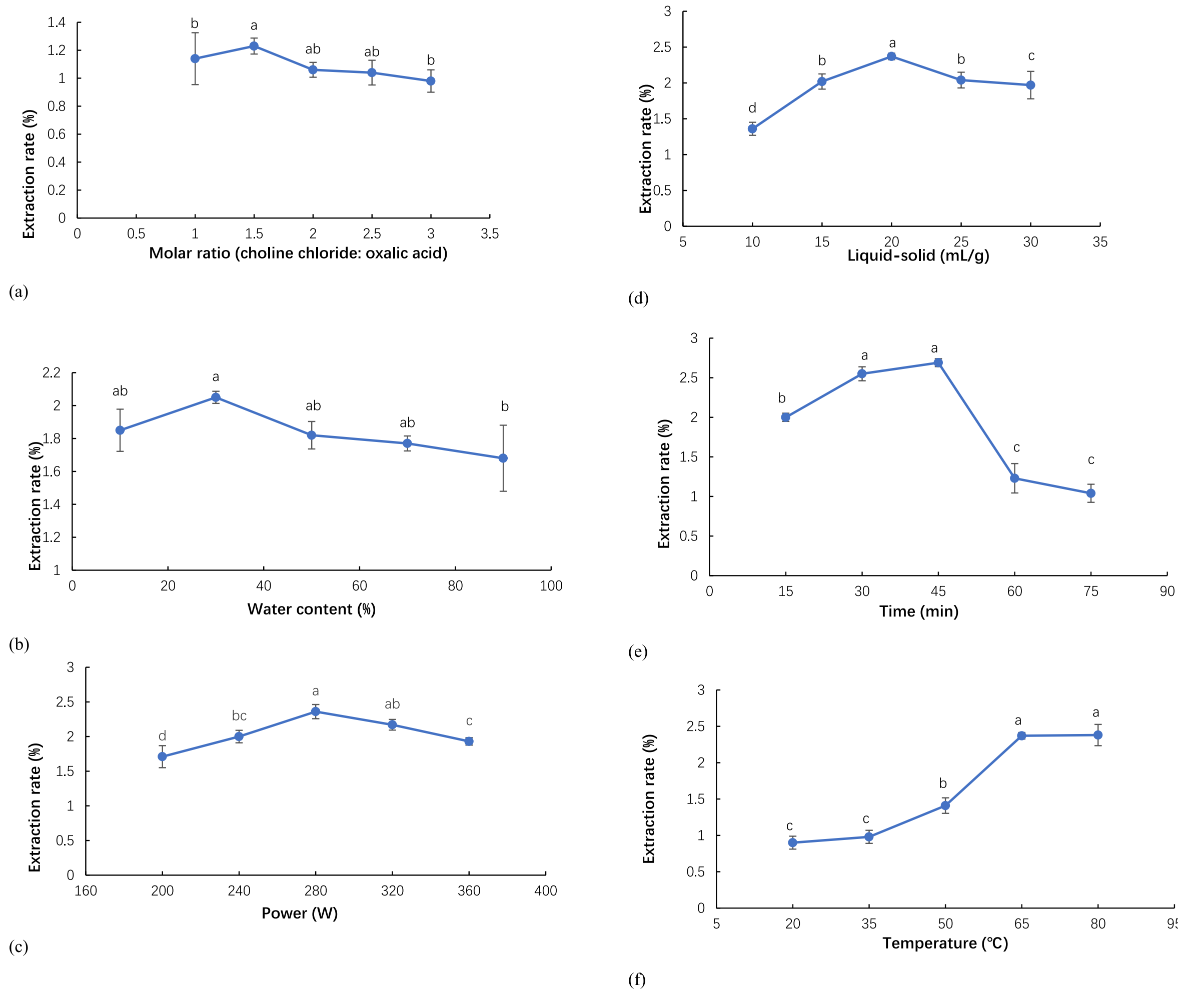

Fig. 2. Different molar ratios of DES (a), water content (b), ultrasonic power (c), liquid-solid ratio (d), ultrasonic time (e), and temperature (f) on the extraction rate of polysaccharides from *Perilla frutescens*.

under otherwise optimal conditions. As shown in Fig. 2d, the polysaccharide extraction yield increased within the range of 10–20 mL/g, but decreased beyond a ratio of 20 mL/g.

3.3.5. *Influence of extraction time*

To determine the optimal extraction time, experiments were conducted over a range of durations (15, 30, 45, 60, and 75 min) while keeping other conditions constant. As presented in Fig. 2e, the polysaccharide extraction yield increased with time and reached a maximum at 45 min, beyond which a decline was observed.

3.3.6. *Influence of temperature*

To evaluate the effect of temperature on extraction efficiency, experiments were conducted at different levels (20, 35, 50, 65, and 80 °C) while keeping other parameters constant. As shown in Fig. 2f, the extraction yield remained relatively stable at lower temperatures but increased noticeably from 65 °C, reaching the highest value at 80 °C.

3.3.7. *Box-Behnken design*

3.3.7.1. Model fitting. Ultrasonic power (A), ultrasonic time (B), ultrasonic temperature (C), and liquid-solid ratio (D) were selected as the four-factor variables affecting the extraction rate of polysaccharides from *P. frutescens*, and the polysaccharide extraction rate was taken as the response value, and the results were statistically analyzed by using a four-factor, three-level design of the experimental scheme (see Table 1).

Table 1
Response surface test factors and levels.

Independent variables	Level		
	−1	0	1
A: Ultrasonic power (W)	240	280	320
B: Ultrasound time (min)	30	45	60
C: Ultrasonic temperature (°C)	50	65	80
D: Liquid- solid ratio (mL/g)	15	20	25

The test results of 29 test points were statistically analyzed using Design-Expert12 software as shown in Table 2. The quadratic multinomial regression equation was obtained as follows: $y = 2.48 + 0.2733A - 0.0542B + 0.0050C + 0.0625D - 0.0775AB - 0.0225AC + 0.0250AD - 0.1825BC - 0.0175BD - 0.1000CD - 0.4090A^2 - 0.4727B^2 - 0.3340C^2 - 0.2453D^2$, where $R^2 = 0.8315$, a fair fit.

According to Table 3, it can be seen that the *P*-value of the model is 0.0023, which is less than 0.05, indicating high significance, and the loss of fit term $P = 0.1299 > 0.05$ is not significant indicating a better fit of the test. The *P* value of the primary term A of the model was < 0.01, and the *P* values of B, C, and D were all > 0.05, indicating that the effect of ultrasonic temperature on the extraction rate was extremely significant, but ultrasonic power, ultrasonic time, and liquid-solid ratio did not have a significant effect on the extraction rate, and the effects of the secondary terms A^2, B^2, C^2, and D^2 on the extraction rate of PFPs were all significant. According to the *F*-value, the influence of each factor on the extraction rate is as follows: temperature > liquid-solid ratio >

Table 2
Box-Behnken design and extraction rate.

Run	Ultrasonic power (W)	Ultrasound time (min)	Ultrasonic temperature (°C)	Liquid-solid ratio (mL/g)	Extraction rate (%)
1	240	45	50	20	1.21
2	240	45	80	20	2.12
3	320	45	50	20	1.24
4	320	45	80	20	1.84
5	280	30	65	15	2.06
6	280	60	65	15	2.04
7	280	30	65	25	1.97
8	280	60	65	25	1.55
9	280	45	50	15	1.58
10	280	45	80	15	1.99
11	280	45	50	25	1.74
12	280	45	80	25	2.25
13	240	30	65	20	1.72
14	320	30	65	20	1.82
15	240	60	65	20	2.02
16	320	60	65	20	1.39
17	280	30	50	20	1.27
18	280	30	80	20	1.74
19	280	60	50	20	1.63
20	280	60	80	20	2.01
21	240	45	65	15	1.41
22	320	45	65	15	1.51
23	240	45	65	25	1.90
24	320	45	65	25	1.93
25	280	45	65	20	2.56
26	280	45	65	20	2.53
27	280	45	65	20	2.45
28	280	45	65	20	2.25
29	280	45	65	20	2.60

Table 3
Quadratic regression model and analysis of variance.

Source	Sum of squares	Df	Mean square	*F*-value	*P*-value	Significant
Model	3.62	14	0.2586	5.06	0.0023	Significant
A	0.8965	1	0.8965	17.55	0.0009	
B	0.0352	1	0.0352	0.6894	0.4203	
C	0.0003	1	0.0003	0.0059	0.9400	
D	0.0469	1	0.0469	0.9178	0.3543	
AB	0.0240	1	0.0240	0.4704	0.5040	
AC	0.0020	1	0.0020	0.0396	0.8450	
AD	0.0025	1	0.0025	0.0489	0.8281	
BC	0.1332	1	0.1332	2.61	0.1286	
BD	0.0012	1	0.0012	0.0240	0.8791	
CD	0.0400	1	0.0400	0.7832	0.3911	
A^2	1.09	1	1.09	21.24	0.0004	
B^2	1.45	1	1.45	28.38	0.0001	
C^2	0.7236	1	0.7236	14.17	0.0021	
D^2	0.3901	1	0.3901	7.64	0.0152	
Residual	0.7150	14	0.0511			
Lack of fit	0.6380	10	0.0638	3.31	0.1299	Not significant
Pure error	0.0771	4	0.0193			
Cor total	4.34	28				

ultrasonic power > ultrasonic time. The optimal extraction conditions were: ultrasonic temperature, power, and time were 70.202 °C, 276.49 W, and 44.955 min, respectively.

3.3.7.2. Box-Behnken design. By using Design-Expert 12 software, the 3D response surface plots and contour plots were obtained after analyzing the results, shown in Fig. 3a–b. The 3D response surface plot represents the interaction of various factors on the response values. The contour plot reflects the influence of interaction between variables. If the shape of the contour plot is an ellipse, it indicates that the influence of the interacting variables is strong. If the shape of the contour plot is a circle, it means the influence is slight. As can be seen in Fig. 3a–b, the extraction rate rises with the enhancement of the corresponding variable and then decreases when variables are further improved. This was consistent with the single factor experiments. The contour plots were ellipses, which confirmed that these interacting variables affected the extraction rate.

3.3.7.3. Validation test results. According to the results of single-factor and response surface experiments and Design Expert software analysis to obtain the optimal extraction conditions: ultrasonic temperature, power, and time were 70.202 °C, 276.49 W, 44.955 min; the liquid-solid ratio was 20.7449 mL/g, the predicted value of polysaccharide extraction of PFPs was 2.53%. To facilitate the experimental operation, the conditions of each factor were adjusted as follows: ultrasonication temperature, power, and time were 70 °C, 280 W, 45 min, the liquid-solid ratio was 21 mL/g, and the polysaccharide yield obtained after the experiment was 2.44%, which was similar to the predicted value. Under these optimized conditions, the polysaccharide yields of water immersion extraction and ultrasonic water extraction were 1.33% and 1.21%, respectively, which were lower than that of the DES extraction method, indicating that the optimization effect was good.

3.3.7.4. Purification results. After the crude polysaccharide was subjected to anion exchange on a DEAE column, an acidic polysaccharide component was obtained, which could be collected by an elution peak named PFP. It appeared in the elution portion of the 0–0.8 M NaCl gradient, and the elution pattern is shown in Fig. 4a. The PFP of the above components was purified and separated by Sephadex G-75 column. Thus, another single elution peak, named PFP-1, was obtained as shown in Fig. 4b. The purity of PFP-1 was calculated to be 84.3% as compared to 32.15% before purification.

3.4. M_w determination

The homogeneity and the M_w of PFP-1 were determined using an HPGPC system. As shown in Fig. 5, a single symmetrical peak was observed in the HPGPC chromatogram of PFP-1, indicating that PFP-1 was a homogeneous polysaccharide. The M_w of PFP-1 was determined to be 11600 Da.

3.5. Results of monosaccharide components

As shown in Fig. 6, the monosaccharide composition of PFP-1 was determined to be rhamnose, glucuronic acid, and glucose, with a molar ratio of 1.45 : 2.82: 1.41. Glucuronic acid was found to be the most abundant component.

3.6. Analysis of bioactivity results

3.6.1. Results of α-glucosidase assay

As shown in Fig. 7a, the inhibitory effect of PFP-1 on α-glucosidase was not strong, and the inhibitory effect on α-glucosidase was much less than that of acarbose. Acarbose had a strong inhibitory effect on α-glucosidase, and it was calculated that the IC_{50} of PFP-1 was 2.386 mg/mL, and the IC_{50} of acarbose was 0.001 mg/mL.

3.6.2. Results of α-amylase experiment

As shown in Fig. 7b, the inhibitory effects of PFP-1 and acarbose on α-amylase were comparable, and the inhibition of α-amylase increased significantly when the concentration of PFP-1 and acarbose increased, with an IC_{50} of 0.002 mg/mL for PFP-1 and 0.003 mg/mL for acarbose.

Therefore, PFP has the potential to become an auxiliary hypoglycemic agent, or even replace traditional hypoglycemic agents to make up for their defects, and has good prospects for development and

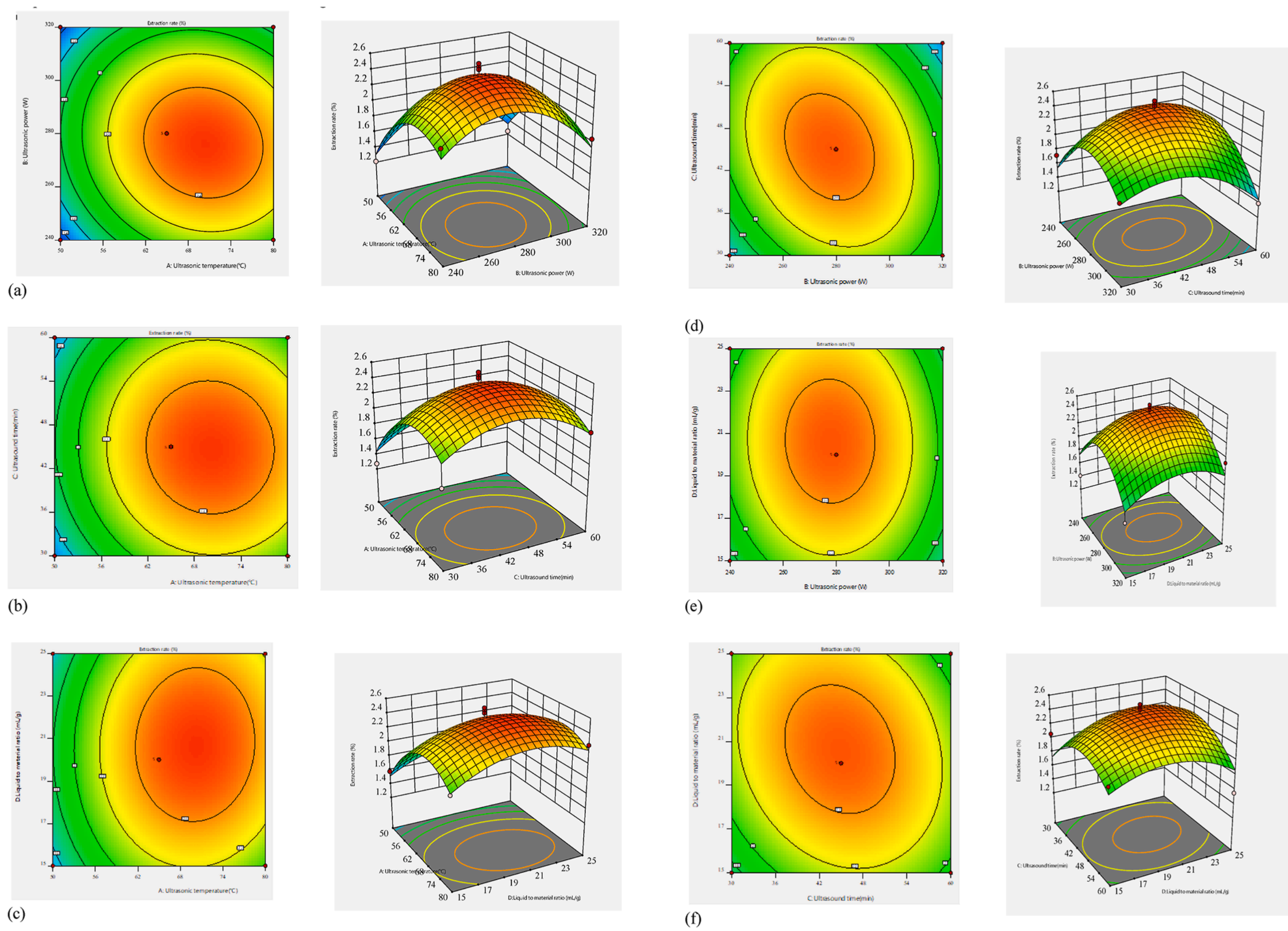

Fig. 3. 3D response surface plots and contour plots showing the effect of ultrasonic temperature and ultrasonic power (a), ultrasonic temperature and ultrasonic time (b), ultrasonic temperature and liquid-solid ratio (c), ultrasonic power and ultrasonic time (d), ultrasonic power and liquid-solid ratio (e), ultrasonic time and liquid-solid ratio (f) on the extraction rate of PFPs.

application. The results of this experiment may be affected by the purity of PFP, resulting in its poor activity of inhibiting α-glucosidase and α-amylase. Therefore, further research is urgently needed.

3.6.3. *DPPH• free radical scavenging results*

As shown in Fig. 7c, the DPPH• radical scavenging ability of PFP-1 increased with concentration and showed a similar trend to that of VitC. The IC_{50} values were calculated to be 0.117 mg/mL for PFP-1 and 0.02 mg/mL for VitC.

3.6.4. *ABTS•$^{+}$ free radical scavenging results*

As shown in Fig. 7d, the ABTS·$^{+}$ radical scavenging activity of PFP-1 was considerably lower than that of VitC. The IC_{50} value was determined to be 0.916 mg/mL for PFP-1 and 0.002 mg/mL for VitC.

4. Discussion

The optimal extraction efficiency at the 1.5:1 M ratio can be attributed to the favorable physicochemical properties of the DES—such as fluidity, viscosity, density, and polysaccharide dissolution capacity—which collectively enhance extraction performance.[23] The decline in yield at higher choline chloride ratios may result from altered solvent characteristics, including increased viscosity or reduced solubility, which impede mass transfer and solvation efficiency. The maximal extraction efficiency at 30% hydration suggests an optimal balance in solvent properties, where moderate polarity and reduced viscosity enhance solvation dynamics and mass transfer of polysaccharides.[24] The decline in yield at higher aqueous fractions may be attributed to altered solvent-solute interactions, increased diffusional resistance, and potential dilution effects, collectively reducing extraction efficiency in hyperhydrated systems. The initial improvement in extraction yield with increasing power can be attributed to enhanced solvent penetration into cellular structures and improved polysaccharide solubilization, facilitated by intensified cavitation and mechanical effects.[25] The decline beyond 280 W may be due to excessive ultrasonic energy leading to high-intensity vortex formation and possible degradation of polysaccharides. Such structural alterations could reduce extractability, indicating that optimal power balances efficient cell disruption with preservation of polysaccharide integrity. The increase in yield with higher solvent volume up to 20 mL/g is likely due to improved solubilization of polysaccharides, as insufficient solvent may lead to incomplete extraction. However, beyond this optimal point, excessive dilution appears to reduce extraction efficiency. This decline may be attributed to a reduction in the driving force for mass transfer and decreased interaction between the solvent and solute, despite initial viscosity limitations being alleviated.[26] Thus, a liquid-solid ratio of 20 mL/g was selected for further experiments to maximize extraction yield while maintaining process efficiency. The initial increase in extraction yield with time can be attributed to enhanced dissolution and diffusion of

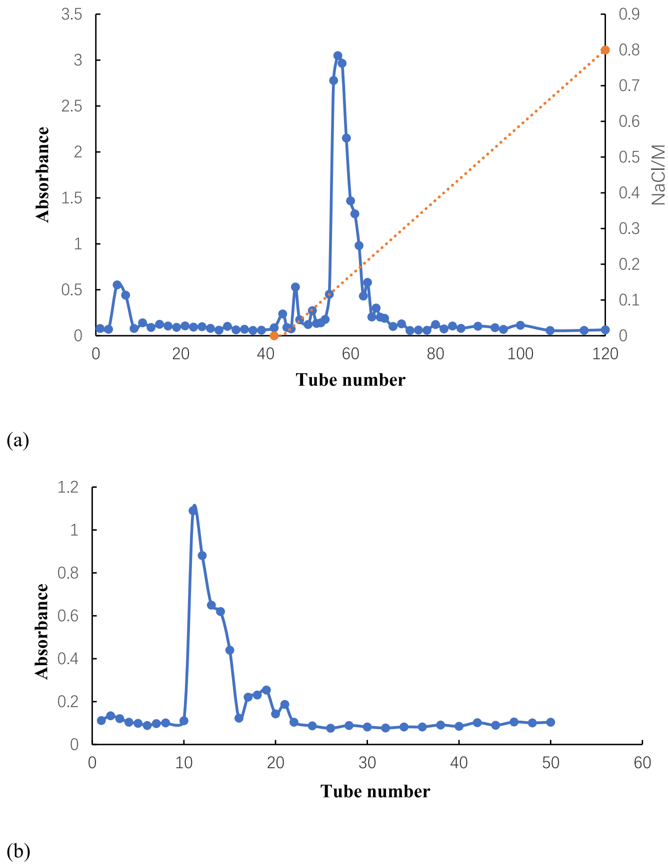

Fig. 4. Elution profiles of PFP on DEAE-Sepharose column (a) and PFP-1 on Sephadex G-75 column (b).

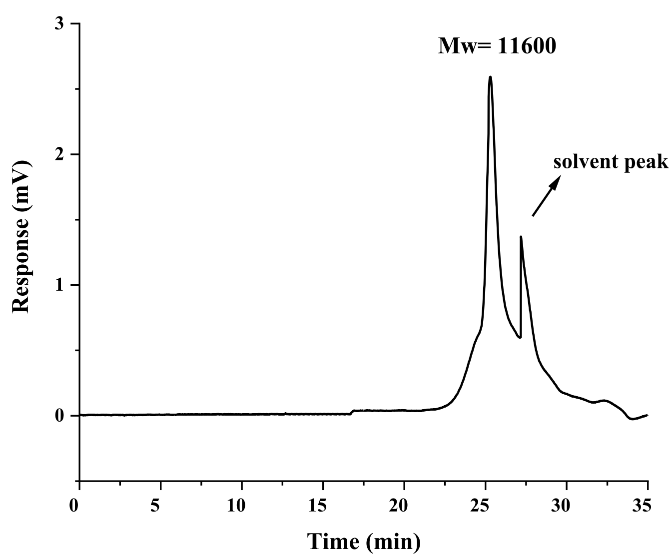

Fig. 5. HPGPC chromatogram of PFP-1.

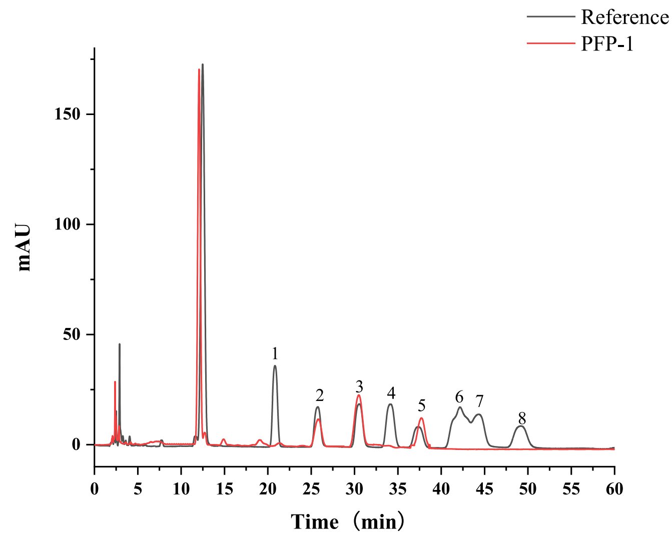

Fig. 6. HPLC chromatogram of nine monosaccharide standards and PFP-1.

polysaccharides, allowing more intense interaction between the solvent and *perilla* powder.[27] However, the decrease beyond 45 min may result from potential degradation of polysaccharides under prolonged exposure to extraction conditions or the possible re-adsorption of dissolved compounds. Thus, 45 min was identified as the optimal duration to achieve maximum yield while maintaining polysaccharide integrity. The improvement in extraction efficiency at elevated temperatures can be attributed to a reduction in solvent viscosity and enhanced dissolution and diffusion of polysaccharides, as supported by L. Zhang & M. Wang.[28] Although the highest yield was achieved at 80 °C, a temperature of 65 °C was selected for subsequent experiments to preserve the structural integrity of PFP and avoid potential thermal degradation.

Monosaccharide composition analysis serves as a fundamental step in polysaccharide characterization. The distinct composition observed in this study offers valuable insights for further elucidating the chemical structure of PFP, which holds significant potential as a dietary supplement. Understanding its structural features is essential for correlating with its physicochemical properties and biological functions, thereby facilitating better application. It is noteworthy that the monosaccharide profile obtained herein differs from that reported by Kim et al.,[29] who identified arabinose, xylose, mannose, glucose, and galactose as the main components of a polysaccharide (PFB-1-0-ii) isolated from the leaves of *P. frutescens*. These discrepancies may arise from variations in extraction methods, plant parts, growing conditions, or polysaccharide fractions.

The concentration-dependent antioxidant behavior and relatively low IC_{50} value demonstrate that PFP-1 possesses notable DPPH• radical scavenging activity, although it remains less potent than VitC. This suggests that *P. frutescens* polysaccharides have potential as natural antioxidants. Comparable findings were reported by H. Zhang et al.,[11] where polysaccharides from perilla seed meal (PSMP) also exhibited strong DPPH• radical scavenging activity, exceeding 75% at concentrations above 2000 μg/mL. The observed antioxidant properties may be attributed to the structural features of the polysaccharides, such as glycosidic linkage patterns, monosaccharide composition, and presence of acidic groups, which can facilitate hydrogen donation and stabilize radicals.

The significantly higher IC_{50} value of PFP-1 compared to VitC indicates its relatively weak ABTS$\bullet^{+}$ radical scavenging capacity. Nevertheless, the polysaccharide still demonstrates measurable antioxidant behavior. These findings are consistent with previous research by H. Zhang et al.,[11] in which ultrasound-extracted PSMP showed 91.10% ABTS radical scavenging activity at 600 μg/mL. The differences in scavenging efficiency may be influenced by structural features such as molecular weight, monosaccharide composition, and functional groups. Although PFP-1 is less potent than VitC, it remains a candidate for further exploration as a natural antioxidant agent.

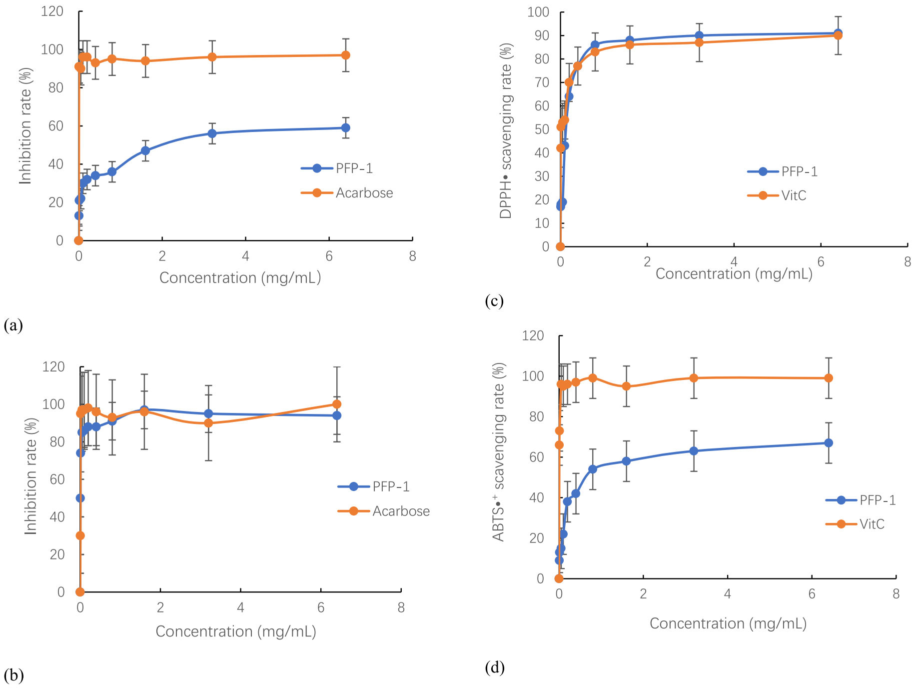

Fig. 7. α-Glucosidase inhibitory activity (a), α-amylase inhibitory activity (b), DPPH• scavenging activity (c), and ABTS•$^+$ scavenging activity (d) of PFP-1.

5. Conclusion

In this study, ultrasound-assisted DES extraction of *P. frutescens* polysaccharides was used, and the optimal extraction conditions were determined as follows: ultrasonic temperature of 70 °C, ultrasonic power of 280 W, ultrasonic time of 45 min, liquid-solid ratio of 21 mL/g, molar ratio of 1.5:1, and moisture content of 30%, which resulted in polysaccharide extraction of 2.44%, and the polysaccharide extraction of *P. frutescens* polysaccharides obtained by the water-soaking method and the aqueous extraction method were 1.33% and 1.21%, respectively, indicating that this experiment has been optimized well. This indicates that the optimization effect of this experiment is good. After the elution of the DEAE column and Sephadex G-75 column, one peak group was obtained, respectively, and the purity of purified polysaccharide was 84.3%. The bioactivity study showed that *P. frutescens* polysaccharide has good antioxidant activity and hypoglycemic activity, and the IC_{50} of *P. frutescens* polysaccharide for DPPH• is 0.117 mg/mL, which is higher than that of VitC (0.02 mg/mL), and the IC_{50} for α-amylase is 0.002 mg/mL, which is lower than that of acarbose (0.003 mg/mL). The M_w of PFP-1 is approximately 11600 Da. After experimental analysis, the monosaccharide composition of PFP-1 was obtained as rhamnose, glucuronic acid, and glucose, with a molar ratio of 1.45: 2.82: 1.41. The structural analysis and the mechanism of hypoglycemic and antioxidant activities of PFP-1 need to be further investigated. It is hoped that more in-depth and diverse reports on the preparation, structural analysis, and activity of PFP will be available in the future.

CRediT authorship contribution statement

Jiajian Tang: Writing – original draft, Methodology. **Zilin Cong:** Writing – review & editing, Writing – original draft, Project administration. **Huijia Dai:** Writing – original draft, Investigation, Formal analysis. **Sigui Zhou:** Funding acquisition. **Yao Wen:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors declared that they have no conflicts of interest to this work.

References

1. Zhu L, Guan LJ, Wang KL, et al. Recent trends in extraction, purification, structural characterization, and biological activities evaluation of *Perilla frutescens* (L.) Britton polysaccharide. *Front Nutr.* 2024;11:1359813.
2. Li H, Zhang H, Zhang Z, et al. Optimization of ultrasound-assisted enzymatic extraction and *in vitro* antioxidant activities of polysaccharides extracted from the leaves of *Perilla frutescens*. *Food Sci Technol.* 2020;40(1):36–45.
3. Kwon KH, Kim KI, Jun WJ, et al. *In vitro* and *in vivo* effects of macrophage-stimulatory polysaccharide from leaves of *Perilla frutescens* var. *crispa*. *Biol Pharm Bull.* 2002;25(3):367–371.
4. Hu Q, Huang G, Huang H. Extraction, structure, activity and application of konjac glucomannan. *Ultrason Sonochem.* 2025;116:107315.
5. Ruesgas-Ramón M, Figueroa-Espinoza MC, Durand E. Application of deep eutectic solvents (DES) for phenolic compounds extraction: overview, challenges, and opportunities. *J Agr Food Chem.* 2017;65(18):3591–3601.
6. Morozova OV, Vasil'eva IS, Shumakovich GP, et al. Deep eutectic solvents for biotechnology applications. *Biochemistry-Moscow.* 2023;88(1):S150–S175.
7. Deng L, Huang G. Ultrasound-assisted extraction, optimization, characteristics and antioxidant activity of *Piper nigrum* L. polysaccharides. *Ultrason Sonochem.* 2025;116:107309.
8. Tang Z, Huang G, Huang H. Ultrasonic-assisted extraction, analysis and properties of purple mangosteen scarfskin polysaccharide and its acetylated derivative. *Ultrason Sonochem.* 2024;109:107010.

9. Wang H, Deng L, Huang G. Ultrasound-assisted extraction and value of active substances in Muxu. *Ultrason Sonochem*. 2025;113:107220.
10. Song ZY, Xiong X, Huang GL. Ultrasound-assisted extraction and characteristics of maize polysaccharides from different sites. *Ultrason Sonochem*. 2023;95:106416.
11. Zhang H, Li H, Zhang Z, et al. Optimization of ultrasound-assisted extraction of polysaccharides from *perilla* seed meal by response surface methodology: characterization and *in vitro* antioxidant activities. *J Food Sci*. 2021;86(2):306–318.
12. Wang H, Huang G, Zhang X. Analysis and properties of polysaccharides extracted from *Brassica oleracea* L. var. *capitata* L. by hot water extraction/ultrasonic-synergistic enzymatic method. *Ultrason Sonochem*. 2025;114:107244.
13. Yue F, Zhang J, Xu J, et al. Effects of monosaccharide composition on quantitative analysis of total sugar content by phenol-sulfuric acid method. *Front Nutr*. 2022;9:963318.
14. Li J, Chen Z, Shi H, et al. Ultrasound-assisted extraction and properties of polysaccharide from *Ginkgo biloba* leaves. *Ultrason Sonochem*. 2023;93:106295.
15. Yao J, Zeng J, Tang H, et al. Effect of deep eutectic solvent extraction on *Auricularia auricula* polysaccharide solubilization and antioxidant potential. *Sustain Chem Pharm*. 2023;34:101166.
16. Tan JQ, Cui PS, Ge SQ, et al. Ultrasound assisted aqueous two-phase extraction of polysaccharides from *Cornus officinalis* fruit: Modeling, optimization, purification, and characterization. *Ultrason Sonochem*. 2022;84:105966.
17. Bian ZY, Zhang R, Zhang X, et al. Extraction, structure and bioactivities of polysaccharides from *Rehmannia glutinosa*: a review. *J Ethnopharmacol*. 2023;305:116132.
18. Dai J, Wu Y, Chen SW, et al. Sugar compositional determination of polysaccharides from *Dunaliella salina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. *Carbohydr Polym*. 2010;82(3):629–635.
19. Jiang F, Sheng Y, Wang F, et al. Characterization and biological activity of acidic sugarcane leaf polysaccharides by microwave-assisted hot alkali extraction. *Food Biosci*. 2023;54:102852.
20. Zhao MM, Bai JW, Bu XY, et al. Characterization of selenized polysaccharides from *Ribes nigrum* L. and its inhibitory effects on α-amylase and α-glucosidase. *Carbohydr Polym*. 2021;259:117729.
21. Chen F, Huang G. Antioxidant activity of polysaccharides from different sources of ginseng. *Inter J Biol Macromol*. 2019;125:906–908.
22. Rozi P, Abuduwaili A, Mutailifu P, et al. Sequential extraction characterization and antioxidant activity of polysaccharides from *Fritillaria pallidiflora* Schrenk. *Inter J Biol Macromol*. 2019;131:97–106.
23. Chen Z, Liu Y, Wang D, et al. Preparation, chemical structure and α-glucosidase inhibitory activity of sulfated polysaccharide from *Grifola frondosa*. *J Funct Food*. 2022;98:105289.
24. Wu DT, Feng KL, Huang L, et al. Deep eutectic solvent-assisted extraction, partially structural characterization, and bioactivities of acidic polysaccharides from lotus leaves. *Foods*. 2021;10(10):2330.
25. Hu XT, Xu FR, Li JL, et al. Ultrasonic-assisted extraction of polysaccharides from coix seeds: optimization, purification, and *in vitro* digestibility. *Food Chem*. 2022;374:131636.
26. Zhang Y, He L, Li Q, et al. Optimization of ultrasonic-assisted deep eutectic solvent for the extraction of polysaccharides from *Indocalamus tessellatus* leaves and their biological studies. *Sustain Chem Pharm*. 2022;30:100855.
27. Tang ZJ, Wang YR, Huang GL, et al. Ultrasound-assisted extraction, analysis and antioxidant activity of polysaccharide from the rinds of *Garcinia mangostana* L. *Ultrason Sonochem*. 2023;97:106474.
28. Zhang L, Wang M. Optimization of deep eutectic solvent-based ultrasound-assisted extraction of polysaccharides from *Dioscorea opposita* Thunb. *Int J Biol Macromol*. 2017;95:675–681.
29. Kim YJ, Hwang JS, Kwon KH. Extraction purification and macrophage stimulatory activity of polysaccharide isolated from leaves of *Perilla frutescens* Britton Var. *Crispa Prog Nutr*. 2019;21:399–405.