Applied Biochemistry and Biotechnology
https://doi.org/10.1007/s12010-024-04990-8

ORIGINAL ARTICLE

Optimization of Ultrasound-Assisted Deep Eutectic Solvent Extraction, Characterization, and Bioactivities of Polysaccharide from *Pericarpium Citri Reticulatae*

Yao Wen[1] · **Jiawen Chen**[1]

Accepted: 5 June 2024

Abstract
The ultrasonic-assisted deep eutectic solvent method was used to extract the polysaccharides of *Pericarpium Citri Reticulatae* (PCRP), and the ultrasound-assisted DES extraction process was optimized by Box-Behnken response surface test using the extraction rate of the PCRP as an index; the in vitro activities of purified the PCRP(PCRPs-1) were investigated by determining the scavenging rate of DPPH• and ABTS•$^{+}$ as well as by enzyme inhibition assay. The monosaccharide composition was analyzed by HPLC. The best process conditions for response surface optimization were a material-liquid ratio of 1:37 g/mL, water content of 44%, time of 89 min, and power of 320 W. The polysaccharide extraction rate was measured to be 5.41%, which was well optimized when compared with that of the ordinary aqueous extraction method of 3.92%. By α-glucosidase and α-amylase inhibition activity test, it showed that the PCRPs-1 had hypoglycemic activity. The DPPH radical scavenging activity test and ABTS $^{+}$ scavenging activity test indicated that the PCRPs-1 had good biological activity. Analysis of the monosaccharide fractions showed that the PCRPs-1 consisted of mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose, and arabinose, with molar ratios of 1:39.24:4.41:8.91:7.83:86.00:1.02:9 .17. The activity studies showed that PCRPs-1 possessed certain hypoglycaemic and antioxidant activities.

Keywords *Pericarpium Citri Reticulatae* polysaccharide · Deep eutectic solvent · Response surface · Biological activity · Monosaccharide composition

Introduction

Pericarpium Citri Reticulatae (PCR), or orange peel, is the ripe fruit peel of the orange and its cultivated varieties in the Brassicaceae family. PCR has a long history of use and high medicinal value, with cardiotonic, anti-shock, anti-tumor, anti-oxidation, and other pharmacological activities. Pericarp contains flavonoids, alkaloids, volatile oils, polysaccharides,

✉ Yao Wen
wenyao169@163.com

[1] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China

and other biologically active substances, these active ingredients have such medicinal values as antioxidant, anti-inflammatory and anti-bacterial, anti-tumor, respiratory protection, cardiovascular protection, and so on. The active ingredients have medicinal values such as antioxidant, anti-inflammatory, anti-bacterial, anti-tumor, respiratory, cardiovascular, and so on [28].

In recent years, some researchers found that under certain conditions of mixing choline chloride and organic reagents, it can form a transparent liquid through hydrogen bonding, and called it deep eutectic solvent (DES), which has the characteristics of high efficiency, degradability, non-pollution, etc., and can dissolve a variety of substances, including polysaccharides, alkaloids, flavonoids and so on [1]. Compared with traditional solvents and ionic liquids, low-eutectic solvents have the advantages of easy preparation, non-toxicity, high thermal stability, high biocompatibility, high extraction rate, and high economic benefits, which have unique advantages in natural plant extraction and separation [5]. The results of the past study showed that the addition of DES can be a sustainable and effective solution for the extraction of natural products, and the extraction method is simple, efficient, environmentally friendly, green, and energy-saving, and can maintain the original properties of polysaccharides [25]. It is noteworthy that DES has also been used to extract various polysaccharides from plants with higher extraction rates than conventional extraction solvents [20, 21]. Thus, all the data suggest that DES has good potential for efficient extraction of polysaccharides from edible and medicinal plants.

However, to the best of our knowledge, the potential applicability of DES in the extraction of bioactive polysaccharides from *Pericarpium Citri Reticulatae* (PCRP) has not been investigated so far. And low-eutectic solvents are still in the development stage, this experiment uses low-eutectic solvents to extract PCRP, and further in-depth study of low-eutectic solvents, exploring suitable types of low-eutectic solvents and broadening the scope of application, which is of far-reaching significance for the research and application of polysaccharide extraction process.

In summary, the search for drugs and natural products with hypoglycemic and antioxidant activities are two hot research topics in the field of medicine. The PCRP has these two biological activities and is highly valuable for application and development. Therefore, the purpose of this study was to explore the optimal extraction conditions of PCRP with DESs through a single-factor experiment and response surface optimization experiment. Also, it aimed to study the antioxidant activity and hypoglycemic activity of PCRP in vitro and determine the monosaccharide composition of the polysaccharide, to provide a basis for the improvement and optimization of industrial extraction, healthcare, and food applications of PCRP.

Materials and Methods

Materials and Reagents

PCR was obtained from Anhui, China. Voucher specimens were deposited in the School of Pharmacy at Guangdong Pharmaceutical University. SephadexG-75 and DEAE-Sepharose were acquired from Lanxiao Technology and New Materials Co., Ltd. (Xian, China). Acarbose and p-nitrophenyl-α-d-glucopyranoside (PNPG) were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China), Alpha-glucosidase, ABTS (2, 2-diazo-di-3-ethylbenzothiazolin-6-sulfonic acid), D-(+) -anhydrous glucose, rhamnose,

D-(+) -galacturonic acid, D-galactose, and D-glucuronic acid were obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). α-amylase, DPPH (1, 1-diphenyl-3-nitrophenylhydrazine), and PMP(1-phenyl-3-methyl-5-pyrazolone) were purchased from Maclin Biochemical Technology Co., Ltd. (Shanghai, China). DNS reagent was bought from Feijing Biotechnology Co., Ltd. (Fuzhou, China). L-arabinose, D-mannose, D-(+) -xylose, and L-(-) -fucose were provided by Desi Biotechnology Co. Ltd. (Chengdu, China). All other chemicals and solvents were of analytical grade.

Preparation of Standard Curves

It was slightly modified according to the reported method [29]. Four milligrams of anhydrous glucose was weighed and prepared as 0.4 mg/mL standard solution. Ten microliters, 20 μL, 30 μL, 40 μL, and 50 μL of standard solution were pipetted into ep tubes and topped up to 100 μL with distilled water, 0.1 mL of 5% phenol and 0.5 mL of concentrated sulfuric acid were added, shaken and allowed to stand for 15 min, three times in parallel. Two hundred microliters of the reaction solution was taken to the spot plate and the absorbance was measured at 492 nm using an enzyme counter. Two hundred microliters of the reaction solution was taken to a spot plate and the absorbance was measured at 492 nm using an enzyme meter and the standard curve was plotted.

Determination of the Extraction Rate of PCRP

The extraction process was slightly modified according to an article [10]. 0. 5 g of PCR powder was weighed, low eutectic solvent was added according to the corresponding material-liquid ratio, mixed well, extracted the PCRP in an ultrasonic cleaner under the corresponding conditions, centrifuged, the filtrate was taken, concentrated, 95% ethanol was added to adjust the concentration while stirring rapidly with a glass rod until the concentration of the alcohol meter showed 80%, refrigerated for 12 h, centrifuged for 5 min at 4000 r/min, and the precipitate was removed by the Sevag method. Proteins were removed by the Sevag method (polysaccharide solution: polysaccharide solution: dichloromethane: n-butanol = 25:4:1) and the precipitate was discarded. The supernatant was discarded and the proteins were removed by the Sevag method (polysaccharide solution: dichloromethane: n-butanol = 25:4:1) and the precipitate was discarded after centrifugation at 4000 r/min for 5 min to obtain the crude polysaccharide of Perilla reticulata solution.

Twenty microliters of PCR crude polysaccharide solution was pipetted into an EP tube, diluted for a certain number of times, take 0.1 mL of dilution, add 0.1 mL of 5% phenol solution, and then add 0.5 mL of concentrated sulfuric acid, shaking, and left to stand for 15 min, take 200 μL of the reaction solution spot plate, absorbance at the wavelength of 492 nm, and measure in parallel for 3 times, and then calculate the extraction rate of PCRP on the basis of the standard curve according to the formula below:

$$\text{Polysaccharide extraction rate} = \frac{C \cdot N \cdot V}{\mathrm{W} \times 1000} \times 100\%$$

where C mass concentration, mg/mL; N dilution factor; V solvent volume, mL; W: herb mass, g

Screening of Deep Eutectic Solvents

According to the method [26] with slight modification, 7 groups of different DESs were prepared. Choline chloride was mixed with oxalic acid, 1, 4-butanediol, malonic acid, urea, citric acid, glycerol, and butanediol in proportion, and dissolved in a water bath at 80℃ to obtain a clear mixture. All the solids were melted into a homogeneous and transparent liquid. Deep eutectic solvents of different compositions were shown in Table 1. The extraction yield of PCRP served as an index, and a group that achieved the highest yield of polysaccharides was selected for the subsequent experiment.

One-Way Tests

DES molar ratio, material-liquid ratio, water content, ultrasound time, ultrasound temperature, and ultrasound power were selected as single factors for the study of extracting PCRP.

(1) Effect of ultrasound time

0.5 g of PCR powder was accurately weighed into several portions and added into the PCR powder with the material-liquid ratio of 1:30 g/mL according to the experimental method of 2.3. The ultrasonic time was set from 10 to 110 min, and the ultrasonic temperature was adjusted to 60℃, and the ultrasonic power was 240 W.

(2) Effect of ultrasound temperature

0.5 g of several portions of PCR powder was accurately weighed and added according to the experimental method of 2.3 with a material-liquid ratio of 1:30 g/mL, an ultrasonic temperature set from 20 to 80℃, an ultrasonic power of 240 W, and an ultrasonic time at the optimal value selected from the above experiments.

(3) Effect of ultrasound power

0.5 g of PCR powder was accurately weighed into several portions and added into PCR powder with a material-liquid ratio of 1:30 g/mL according to the experimental method in 2.3. The ultrasonic power was set at 160 to 360 W, and the ultrasonic time and ultrasonic temperature were the optimal values selected in the above experiments.

(4) Influence of material-liquid ratio

0.5 g portions of PCR powder were weighed, and according to the experimental method of 2.3, the material-liquid ratios from 1:10 to 1:60 g/mL were selected, and the ultrasonic time, ultrasonic temperature, and ultrasonic power were the optimal values selected in the above experiments.

Table 1 Deep eutectic solvents of different compositions

DES	Hydrogen bond acceptor	Hydrogen bond donor	Molar ratio (HBA: HBD)	Ultrasound time min	Ultrasonic temperature ℃	Ultrasonic power W	material-liquid ratio g/mL	Moisture content %
DES-1	Choline chloride	oxalic acid	1:1	30	60	240	1:30	30
DES-2	Choline chloride	1,4-butanediol	1:2					
DES-3	Choline chloride	malonic acid	1:2					
DES-4	Choline chloride	urea	1:2					
DES-5	Choline chloride	citric acid	1:1					
DES-6	Choline chloride	glycerol	1:2					
DES-7	Choline chloride	butylene glycol	1:2					

(5) Effect of water content

0.5 g of PCR powder was weighed and the water content was selected from 0 to 90% according to the experimental method in 2.3. The ultrasonication time, ultrasonication temperature, ultrasonication power, and the material-liquid ratio were the optimal values selected in the above experiments.

(6) Effect of molar ratio

Several portions of 0.5 g of PCR powder were weighed, and according to the experimental method of 2.3, oxalic acid: choline chloride was selected as 1:1.5 to 3:1, and ultrasonication time, ultrasonication temperature, ultrasonication power, material-liquid ratio, and water content were the optimal values selected for the above experiments.

Response Surface Design Experiments of PCRP Extraction Process

According to the results of the one-way test, we chose to select four factors that affect the extraction rate of PCRP, ultrasound time (A), water content (D), material-liquid ratio (C), ultrasound power (D) as the response variable, and the extraction rate of PCRP (Y) as the response value, and the levels of the selected factors and their response surface design of the experiment are shown in Table 2.

The Best-Optimized Process for the Extraction of PCRP

0.5 g of PCR powder was weighed precisely, extracted by ultrasonic extraction according to the optimal conditions derived from response surface optimization, centrifuged, the supernatant was collected, concentrated, ethanol precipitated, protein removed, centrifuged, and 0.5 g of the supernatant was taken into an ep tube, and then the color was developed by phenol–sulfuric acid method, and then shaken, and then placed in the room for 15 min at room temperature, and then the absorbance at 492 nm was determined, and then measured in parallel for three times.

Plain Water Extraction

0.5 g of PCR powder was weighed precisely, dissolved with 25 mL of distilled water, dissolved by ultrasonic for 10 min, and the extraction rate was determined by phenol sulfuric acid method. The three experiments were carried out simultaneously.

Table 2 Response surface factor level table

Level (of achievement etc.)	Considerations			
	Liquid–liquid ratio g/mL	Ultrasound time min	Ultrasonic power W	Moisture content %
-1	10	50	280	30
0	30	70	320	40
+1	50	90	360	50

Isolation and Purification of Crude Extracts of PCRP

Loading of DEAE-Sepharose Fast Flow Fiber Columns

It was slightly modified according to the method [22]. After rinsing the packing DEAE with distilled water until no alcohol remains, add a small amount of distilled water and stir to form a paste. Place the chromatography column (2.5×40) vertically and pour in the packing while opening the screw clamp of the lower tube to make the flow rate of 1.6 mL/min. Be careful that no air bubbles can be generated throughout the process and that the level of distilled water cannot be lower than that of the DEAE. If any of the above occurs, the column should be reassembled. After the DEAE settles naturally, mount the upper end of the column.

SephadexG-75 Dextran Gel Column Loading

A certain amount of gel filler SephadexG-75 was weighed for a boiling water bath and dissolved for 3 h. During this time, it was stirred continuously with a glass rod, and after the boiling water bath, it was left to stand, and the upper layer of the aqueous solution, as well as fine particles, was discarded. The packing was then soaked in distilled water and washed 2 to 3 times. Place the chromatography column (1.5×100) vertically and pour in the packing while opening the screw clamp of the lower tube and adjusting the flow rate to 0.5 mL/min. No air bubbles can be generated during the whole process, and if there are any, it is necessary to reload. After its natural settlement in the column is completed, load the upper end of the column [33].

Isolation and Purification of Crude Polysaccharides

After the crude extract was concentrated in a rotary evaporator, the concentrate was heated and evaporated in a water bath at 60℃ to make an extract, dried to constant weight to obtain PCRP, prepared as 25 mg/mL crude polysaccharide solution (named LPS), centrifuged at 2000 r/min for 10 min, and passed through the membrane to obtain the sample solution as a standby. Eight milliliters of sample solution was taken on a DEAE-Sepharose Fast Flow chromatography column. Distilled water and gradient sodium chloride were used as eluents at a flow rate of 1.6 mL/min, and 8 mL was collected in each tube, 50 tubes were washed with water and 80 tubes were washed with salt. The phenol–sulfuric acid method was used to develop the color and plot the elution curve, and one fraction was collected for each peak.

The collected effluent was concentrated, packed into a dialysis bag (retention capacity of 3500 Da), dialyzed under running water for 24 h, concentrated and dried to prepare 10 mg/mL of sample solution, centrifuged, passed through microporous filter membrane, and 4 mL of the filtrate was taken onto a Sephadex G-75 column, distilled water was used as the eluent at a flow rate of 0.5 mL/min, and 8 mL of the sample was collected from each tube, and 50 tubes were collected. The phenol–sulfuric acid method was used to develop the color and plot the elution curve, and one component was collected for each peak.

Purity Calculations

The sample was obtained by concentrating and drying the eluate collected by G-75, weighing a certain amount of the sample before and after purification adding distilled water to formulate a certain concentration, phenol–sulfuric acid to develop the color, and calculating the purity according to the following formula:

$$\text{Purity}\% = \frac{c * V}{m} \times 100\%$$

where C polysaccharide concentration, mg/mL; V solution volume, mL; m mass of evaporated extract, mg.

Biological Activity Studies

Hypoglycemic Activity

α-Glucosidase Inhibitory Activity Test According to the detection method of Deng and with slight modifications, the experimental scheme for the determination of α-glucosidase inhibitory activity of polysaccharide extract from orange peel was carried out [6]. Different concentrations of sample solutions and acarbose solution (3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 μg/mL, 40 μL) were mixed with α-glucosidase (1 U/mL, 40 μL) in 96-well plate, and the mixture was incubated at 37℃ for 10 min. Twenty microliters of PNPG (3 mM) was added to the mixture and incubated at 37 °C for 30 min. Finally, the reaction was terminated by adding 100 μl Na_2CO_3 solution (1 M). Acarbose was used as a positive control, the absorbance at 405 nm was measured three times in parallel, and the rate of α-glucosidase inhibition was calculated according to the following formula:

$$\alpha - \text{glucosidase inhibition rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%$$

where, A_0: α-glucosidase solution + PNPG solution; A_1: sample solution + α-glucosidase solution + PNPG; A_2: sample solution + PNPG solution.

α-Amylase Inhibitory Activity Test According to the detection method of Yun et al. and slightly modified, the experimental scheme for the determination of α-amylase inhibitory activity of orange peel polysaccharide extract was carried out [30]. Different concentrations of sample solutions and acarbose solution (3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 μg/mL, 40 μL) were mixed with α-amylase (0.5 mg/mL, 40 μL) in ep tubes and incubated at 37℃ for 10 min. Twenty microliters of 1% starch solution was added and incubated at 37 °C for 10 min. The reaction was terminated by adding 100 μL of DNS reagent, incubated at 100 °C for 10 min, and the mixture was diluted with 1 mL of distilled water. Acarbose was used as a positive control, the absorbance at 540 nm was measured three times in parallel, and the α-amylase inhibition was calculated according to the following formula:

$$\alpha - \text{amylase inhibition rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%$$

where, A_0: α-amylase solution + starch solution; A_1: sample solution + α-amylase solution + starch solution; A_2: sample solution + starch solution.

Antioxidant Activity

Measurement of DPPH Radical Scavenging Activity According to the detection method of Zhu et al. and slightly modified, the experimental scheme for the determination of DPPH scavenging activity of orange peel polysaccharide extract was carried out [34]. A series of sample solutions and vitamin C (VitC) reference solutions with different concentrations (3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 μg/mL) were prepared. Each concentration of 100μL sample solution or control solution was mixed with 100 μL DPPH radical solution in a 96-well plate and shaken on a shaker in the dark for 30 min. The absorbance at 517 nm was measured three times in parallel. DPPH clearance was calculated according to the following formula:

$$\text{DPPH Clearance rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%$$

where, A_0: DPPH + distilled water; A_1: sample solution + DPPH; A_2: sample solution + anhydrous ethanol.

ABTS $^+$ Free Radical Scavenging Activity Assay According to the detection method of Mohammed et al. and slightly modified, the experimental scheme for the determination of the scavenging activity of orange peel polysaccharide extract on ABTS$\bullet^+$ was carried out [18]. A series of sample solutions and VitC reference solutions with different concentrations (3200, 600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μg/mL) were prepared. 100 μL of sample solution or control solution of each concentration was mixed with 100 μL of ABTS$\bullet^+$ working solution (mixed with equal volume of 7.4 mM ABTS$\bullet^+$ solution and 2.6 mM potassium peroxodisulfate and diluted with absolute ethyl alcohol until $A_{734nm} = 0.7 \pm 0.02$) in 96-well plate, shaken in the dark on a shaker for 30 min. The absorbance at 734 nm was measured three times in parallel, and the ABTS$\bullet^+$ clearance was calculated according to the following formula:

$$\text{ABTS Clearance rate} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\%$$

where A_0: ABTS $^+$ working solution + anhydrous ethanol; A_1: sample solution + ABTS $^+$ working solution; A_2: sample solution + anhydrous ethanol.

Analysis of the Monosaccharide Fractions of PCRP

The monosaccharide composition of PCRP was analyzed by the PMP pre-column derivatization-HPLC method [15].

PMP Derivatization of Mixed Monosaccharides Standard Samples

The experiment was carried out according to the method [4]. The mixed standard solution of 2 mM was prepared with arabinose (Ara), mannose (Man), xylose (Xyl), fucose (Fuc), galactose (Gal), galacturonic acid (Gal-A), rhamnose (Rha), glucose (Glc) and glucuronic acid (Glc-A). 50 μL of the above standard solution mixed with 450 μL 0.5 M PMP-methanol solution. Then, 450 μL of 0.3 M sodium hydroxide solution was added, mixed, and reacted in a water bath at 70 °C for 30 min. After cooling, 460 μL of 0.3 M hydrochloric acid solution was added to neutralize, 2 mL of dichloromethane was added to extract, the dichloromethane layer was separated, and more than two steps were repeated to remove excess PMP. Finally, the water layer was filtered through a 0.45 μm water system microporous membrane for HPLC analysis.

PMP Derivatization of PSPs-1

Five milligrams of the PSPs was placed in a 10-mL plug tube, and 2 mL 2 M TFA solution was added. After sealing, the polysaccharide was completely hydrolyzed in an oil bath at 120 °C for 6 h. The hydrolysate was transferred to a 50-mL round-bottom flask and concentrated to dryness at 65 °C under reduced pressure. Six milliliters of methanol was added to dry again, repeated three times to remove TFA, and then 800 μL of deionized water was added to dissolve. The derivatization method was the same as above.

Chromatographic Conditions

The analytical instrument was an Agilent high-performance liquid chromatograph with a Kromasil 100–5-C18 column (5 μm, 4.6 × 250 mm), a UV detector (wavelength: 250 nm), a 10 μL injection volume, a mobile phase of 0.05 M phosphate buffer solution (pH 6.7)-acetonitrile (v/v = 85: 15), and a flow rate of 1 mL/min.

Results and Discussion

Results of Standard Curve Plotting

The glucose concentration-absorbance standard curve was plotted, and the regression equation was $A = 3.6643C\text{-}0.0111$, $R^2 = 0.9991$, indicating that the curve was well fitted.

Determination of the Optimum Low Eutectic Solvent System

The effect of low eutectic solvent type on the extraction rate of PCRP is shown in Fig. 1.

From Fig. 1, the highest extraction rate of polysaccharides from peel powder was obtained when the molar ratio of choline chloride to oxalic acid was 1:1, probably because the mixed system of choline chloride and oxalic acid was less viscous and more diffusive, which led to an increase in the polysaccharide solubilization rate [6]. Therefore, the best low eutectic solvent for extracting PCRP was choline chloride and oxalic acid. Different compositions of DESs contributed to varied properties of DESs including the polarity, capability to dissolve natural compounds, viscosity, and mobility. Thus, the extraction rate was governed by these properties and varied when using different DESs [17].

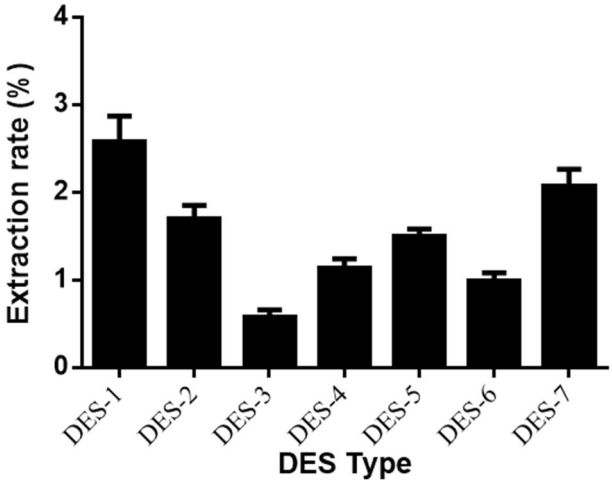

Fig. 1 Different kinds of low eutectic solvents for extracting PCRP

Results and Analyses of One-Way Tests

(1) Effect of ultrasound time

As can be seen from Fig. 2A, the polysaccharide extraction rate reached the maximum value at 70 min, and the extraction rate gradually decreased as the ultrasonic time exceeded 70 min. Part of the reason may be that proper time promoted the diffusion of dissolution of the polysaccharide with intensive interaction between the solvent and the PCR powder [30]. With the increase of ultrasound time, the ultrasound can fully function on the cells of RRT, destroying the cell wall, so the PCRP dissolves more. However, if the ultrasound time was further prolonged, the powerful effect of ultrasound may destroy the polysaccharide, thus reducing the extraction rate [31]. Therefore, the ultrasonication time of 70 min was chosen for the subsequent experiments. Wei Li et al. [12] used the response surface method to determine the best ultrasonic-assisted extraction time of polysaccharides from *Platycodon grandiflorum* [20 min].

(2) Effect of ultrasound temperature

As can be seen from Fig. 2B, the polysaccharide extraction rate reaches the maximum value at 80℃, which is due to the increase in temperature, the molecular thermal movement is intensified, the cell rupture speed is accelerated, accelerating the diffusion of polysaccharides in the solvent. The temperature is too high will cause the polysaccharide molecular structure damage, resulting in a decrease in the polysaccharide extraction rate [31]. Therefore, the ultrasonic temperature of 80 ℃ was selected for the subsequent experiments. Yapeng Li et al. [13] optimized the ultrasonic assisted extraction of Pericarpium Citri Reticulatae "Chachiensis" polysaccharides with an optimal extraction temperature of 90 ℃.

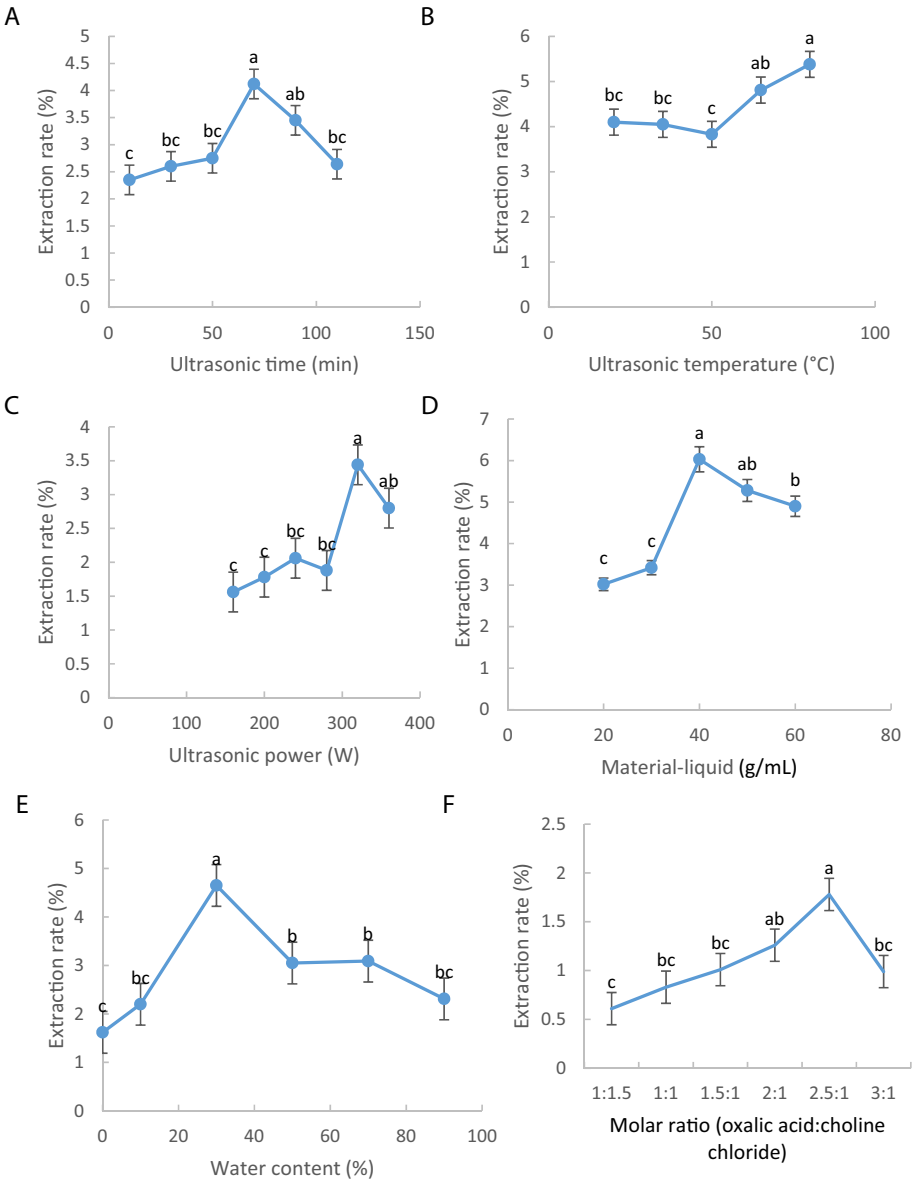

Fig. 2 The effect of different ultrasonic time (**A**), temperature (**B**), ultrasonic power (**C**), material-liquid ratio (**D**), water content (**E**), and molar ratios of DES (**F**) on the extraction rate of PCRP

(3) Effect of ultrasound power

As can be seen from Fig. 2C, with the increase of ultrasonic power, the peak value appeared at 320 W, and after the peak value, the extraction rate showed a decreasing trend. This is due to the increase in ultrasonic power, accelerating the diffusion of polysaccharides in the solvent and improving the polysaccharide extraction rate [8], while the power is too high so that the

structure of polysaccharides is destroyed and the polysaccharide content is reduced [2]. Therefore, the ultrasonic power of 320 W was selected for subsequent experiments. Yapeng Li et al. [13] used the response surface method to optimize the ultrasonic assisted extraction process of Pericarpium Citri Reticulatae "Chachiensis" polysaccharides to determine that the best ultrasonic power was 250 W.

(4) Influence of material-liquid ratio

As can be seen from Fig. 2D, the polysaccharide extraction rate was positively correlated with the material-liquid ratio in the range of 1:20 ~ 1:40 g/mL, which increased with its increase, and the peak appeared at 1:40 g/mL, and the extraction rate showed a decreasing trend after the peak. This is a small amount of solvent in the system that cannot be completely wetted peel powder leads to the dissolution of polysaccharides from the peel is incomplete, while the material-liquid ratio is too large, there are other soluble components in the peel, which will lead to some soluble components are also dissolved, to a certain extent, affecting the dissolution of polysaccharides, and thus the polysaccharide extraction effect is poor [32]. Therefore, the material-liquid ratio of 1:40 g/mL was selected for the subsequent experiments. Yapeng Li et al. [13] used the response surface method to optimize the ultrasonic assisted extraction process of Pericarpium Citri Reticulatae "Chachiensis" polysaccharides to determine the best liquid–solid ratio of 30:1 mL/g.

(5) Effect of water content

As can be seen from Fig. 2E, the polysaccharide extraction rate was positively correlated with water content in the range of 0–30%. Appropriate water content can promote material mass transfer [25]. The extraction rate decreased after the peak. This is due to the natural low eutectic solvent having viscosity, high viscosity will lead to the slow diffusion of solutes in the extraction process, adding a certain amount of water can adjust the viscosity of the low eutectic solvent. Therefore, a water content of 30% was selected for subsequent experiments.

(6) Effect of molar ratio

As can be seen from Fig. 2F, the polysaccharide extraction rate was positively correlated with the molar ratio of oxalic acid: choline chloride in the range of 1:1.5 ~ 2.5:1. When the molar ratio of oxalic acid to choline chloride gradually increased, the viscosity of the natural low eutectic solvent increased, and the extraction rate of PCRP increased. The viscosity and surface tension of DES reached the best value, so the extraction effect is better [3]. When the viscosity reached a certain height, the extraction rate would decrease. And when the molar ratio was 3:1, the extraction rate was at a low level, which is probably because the components were more likely to crystalize at room temperature, thus hindering the dissolution of PCRP [24]. Thus, the molar ratio of oxalic acid: choline chloride 2.5:1 was selected for the subsequent tests.

Response Surface Test Results and Analyses

Based on the results of the one-way test, the results of the response surface test designed according to Box-Behnken's principle with PCR, polysaccharide extraction rate as the response value, and ultrasound time (A), water content (B), material-liquid ratio (C), and ultrasound power (D) as the response variables are shown in Table 3.

The results of 29 test points based on BBD random testing were statistically analyzed using Design Expert 11 software, as shown in Table 3. According to the experimental data obtained by the regression method, the extraction rate of PCRP (Y) and the ultrasonic time (A), water content (B), material-liquid ratio (C), and ultrasonic power (D) can be fitted to the following second-order polynomial equation: $y = 2.92 + 0.20A + 0.85B + 0.59C — 0.32D + 0.58AB — 0.36AC + 0.21AD + 0.24BC - 0.36BD - 0.46CD + 0.35A^2 + 0.012B^2 + 0.56C^2 + 0.44D^2$. where R^2 is equal to 0.8280, a fair fit.

Significance test and ANOVA were performed on the above model and the results are shown in Table 4.

As can be seen from Table 4, the established model for the extraction rate of PCRP was significant ($P < 0.05$) and the difference in the P-value of the misfit error was not significant ($P > 0.05$), which indicated that the model fitted the independent variables well. The

Table 3 Response surface experiment reagents and results

Test Group	Ultrasound time (min)	Moisture content (%)	Material-liquid ratio (g/mL)	Ultrasonic power (W)	Extraction rate (%)
1	50	10	1:40	320	2.9
2	90	10	1:40	320	2.22
3	50	50	1:40	320	2.83
4	90	50	1:40	320	4.46
5	70	30	1:30	280	3.07
6	70	30	1:50	280	5.66
7	70	30	1:30	360	2.74
8	70	30	1:50	360	3.5
9	50	30	1:40	280	4.17
10	90	30	1:40	280	4.02
11	50	30	1:40	360	3.02
12	90	30	1:40	360	3.71
13	70	10	1:30	320	1.64
14	70	50	1:30	320	4.06
15	70	10	1:50	320	2.49
16	70	50	1:50	320	5.86
17	50	30	1:30	320	3.15
18	90	30	1:30	320	4.31
19	50	30	1:50	320	4.4
20	90	30	1:50	320	4.12
21	70	10	1:40	280	2.57
22	70	50	1:40	280	4.44
23	70	10	1:40	360	3.36
24	70	50	1:40	360	3.78
25	70	30	1:40	320	3.01
26	70	30	1:40	320	2.9
27	70	30	1:40	320	2.62
28	70	30	1:40	320	3.32
29	70	30	1:40	320	2.76

interaction term AB was not significant as indicated by the *P* value. From the *F* value, it can be seen that the order of the effect of each factor on the extraction rate of PCRP: water content > material-liquid ratio > ultrasonic power > ultrasonic time.

Figure 3A–F shows the response surface and contour plots, which reflect the results of the response values under the interaction of various factors, using the Design-Expert 11 software. The steeper the response surface or the more elliptical the shape of the contour lines, the more significant the interaction between the two factors [7]. The steeper the response surface or the more elliptical the contour shape, the more significant the interaction between the two factors. The apex of the response surface is the center of the smallest ellipse in the contour.

As shown in Fig. 3A–F, in a certain range, with the increase of various factors, the extraction rate of PCRP gradually increased until the response surface had the maximum value, after increasing various factors its instead gradually decreased.

Determination of Optimum Process Conditions and Validation Test

After the response surface analysis, the optimal parameters were material-liquid ratio 1:37.387 g/mL, water content 44.374%, time 88.864 min, power 326.585 W, and the theoretical value of PCRP was 4.328%. To facilitate the operation, the following adjustments were made: material-liquid ratio 1:37 g/mL, water content 44%, time 89 min, power 320 W. The validation results showed that the yield of PCRP was 5.41%, which was similar to the theoretical extraction rate, and it proved that the model fitting degree was OK. Compared

Table 4 ANOVA results of polysaccharide extraction rate

Source	Sum of Squares	df	Mean Square	*F*-value	*P*-value	
Model	21.46	14	1.53	4.81	0.0029	significant
A-Time	0.47	1	0.47	1.47	0.2454	
B-Moisture content	8.76	1	8.76	27.49	0.0001	
C-liquid ratio	4.15	1	4.15	13.04	0.0028	
D-Power	1.22	1	1.22	3.82	0.0710	
AB	1.33	1	1.33	4.19	0.0599	
AC	0.52	1	0.52	1.63	0.2228	
AD	0.18	1	0.18	0.55	0.4690	
BC	0.23	1	0.23	0.71	0.4141	
BD	0.53	1	0.53	1.65	0.2197	
CD	0.84	1	0.84	2.63	0.1272	
A^2	0.79	1	0.79	2.49	0.1371	
B^2	9.211E-004	1	9.211E-004	2.893E-003	0.9579	
C^2	2.04	1	2.04	6.40	0.0240	
D^2	1.26	1	1.26	3.96	0.0666	
Residual	4.46	14	0.32			
Lack of fit	4.17	10	0.42	5.88	0.0513	not significant
Pure Erro	0.28	4	0.071			
Cor Total	25.92	28				

$P < 0.01$, highly significant effect; $P < 0.05$, significant effect

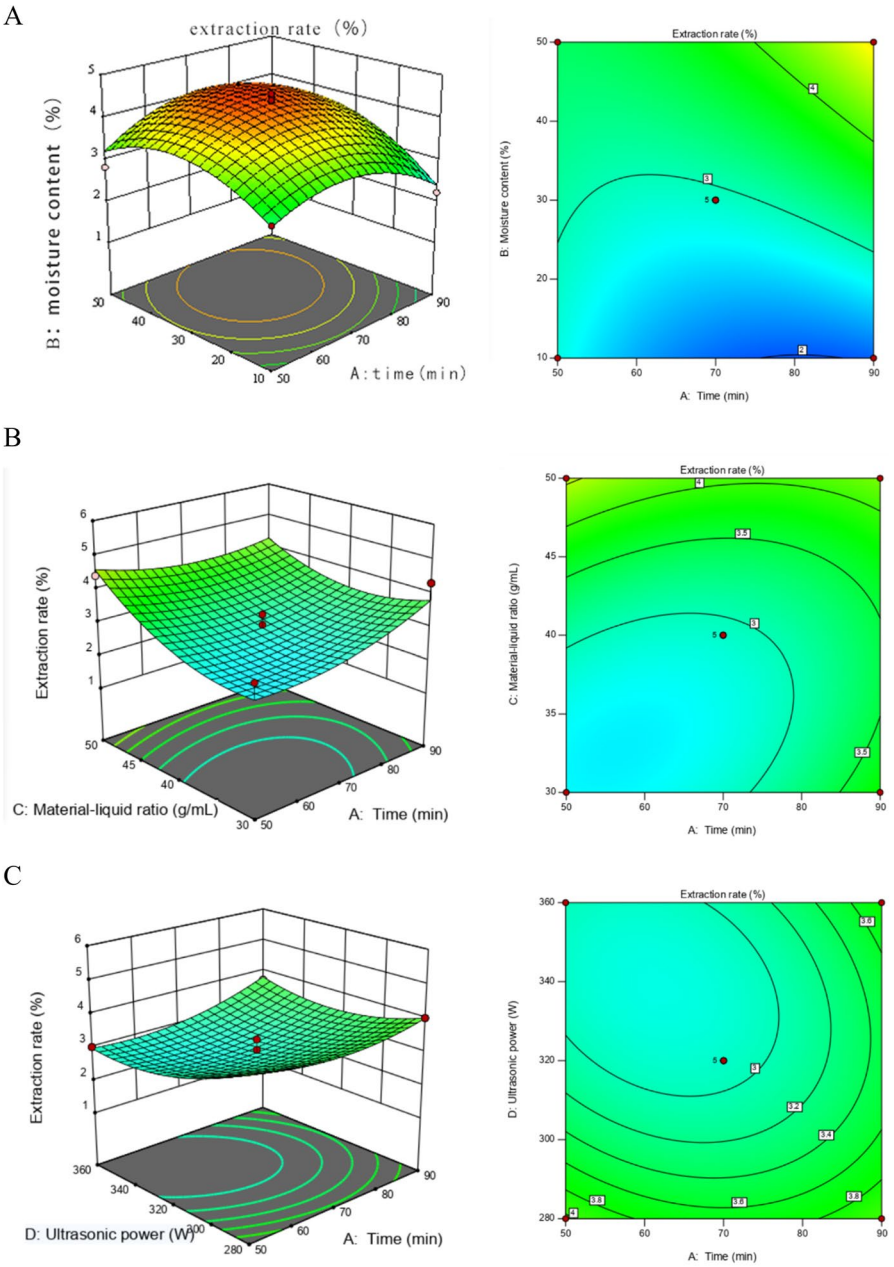

Fig. 3 3D response surface plots and contour plots showing the effect of time and water content (**A**), time and material-liquid ratio (**B**), ultrasonic time and ultrasonic power (**C**), water content and material-liquid ratio (**D**), water content and ultrasonic power (**E**) and ultrasonic power and material-liquid ratio (**F**) on the extraction rate of PCRP

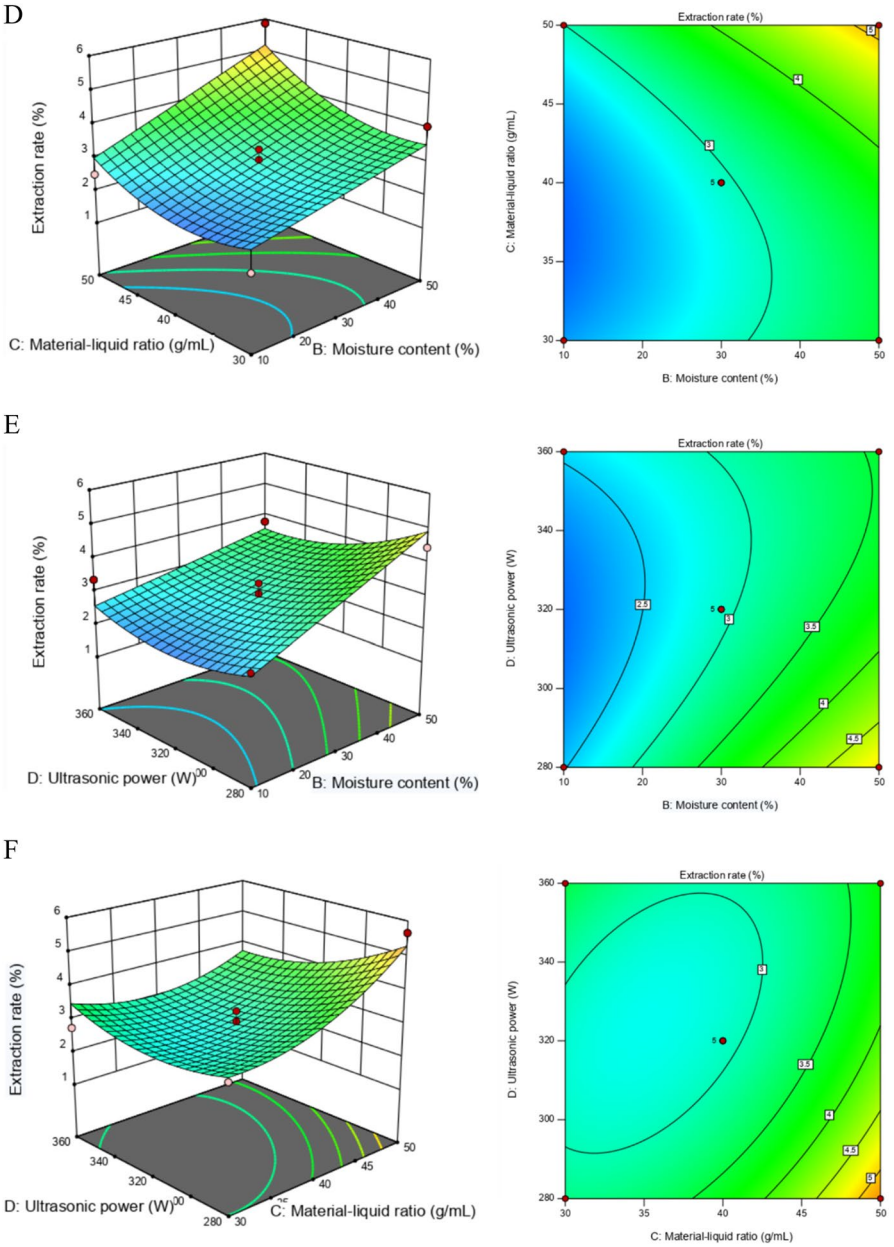

Fig. 3 (continued)

with the extraction rate of 3.82% in the normal water extraction method, the optimization effect was good.

Results and Analysis of the Isolation and Purification of Crude PCRP

After the crude polysaccharides were separated on a DEAE fiber column, one elution peak appeared in the 0 ~ 0.8 M NaCl gradient elution fraction, named PCRPs, and the elution profile is shown in Fig. 4A.

The above-obtained component PCRPs were separated and purified by Sephadex G-75 chromatography column to obtain a single elution peak and named PCRPs-1, the elution profile is shown in Fig. 4B.

Component PCRPs-1 was subjected to phenol–sulphuric acid chromatography and the purity was calculated to be 92.46% and the purity of PCRPs was 71.38%.

Results and Analysis of the Hypoglycemic Activity of PCRPs-1

Diabetes mellitus is a metabolic disease characterized by high blood glucose, in which the metabolism of sugars, fats, and proteins is disrupted due to high blood glucose, resulting in the dysfunction of various tissues and organs. Clinically, it is characterized by persistent hyperglycemia, and typical diabetic patients show the syndrome of "three more and one less," such as polydipsia, polyuria, poly food, weight loss, etc. [27].

Acarbose, a biosynthetic pseudo tetrasaccharide, is an α-glucosidase inhibitor that inhibits α-glucosidase activity in the brush border of cells in the small intestinal wall, prolonging the time for polysaccharides or disaccharides to be catabolized and absorbed in the intestinal tract, and thus effectively lowering blood glucose [14] and, acarbose can inhibit α-amylase activity [19].

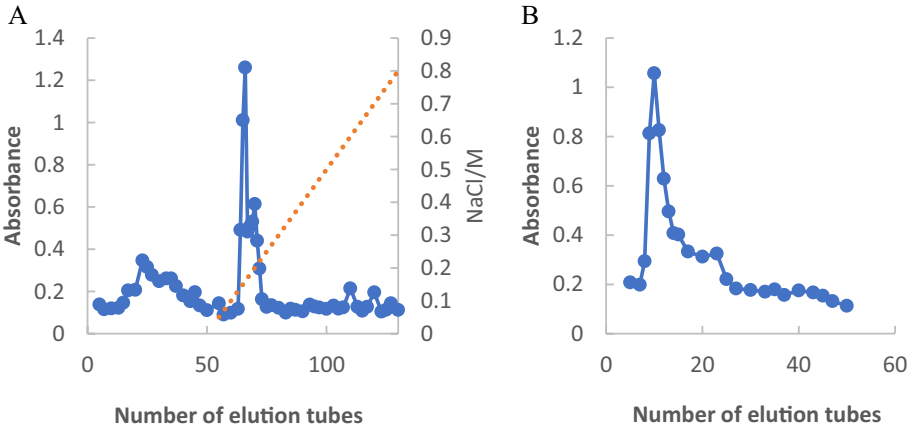

Fig. 4 Elution profile of PCRP (**A**) on DEAE-sepharose column and PCRPs-1 (**B**) on Sephadex G-75 column

α-Amylase Inhibitory Capacity

As shown in Fig. 5A, when the concentrations of PCRPs-1 and acarbose were gradually increased, their inhibitory effects on α-amylase were gradually enhanced. It was calculated that the IC_{50} value of PCRPs-1 was 2.84 mg/mL, which was higher than that of acarbose (0.035 mg/mL), so that PCRP had the effect of inhibiting α-amylase, but its effect was weaker than that of acarbose. Wei Li et al. [11] measured that the IC_{50} value of polysaccharides from *Platycodon grandiflorum* roots against α-amylase (2.59 mg/mL) was lower than that of PCRPs-1 (2.84 mg/mL).

α-Glucosidase Inhibitory Capacity

As shown in Fig. 5B, when the concentrations of PCRPs-1 and acarbose gradually increased, their inhibitory activities against α-glucosidase were increasing. It was calculated that the IC_{50} of PCRPs-1 was 1.82 mg/mL, which was significantly lower than that of acarbose (3.42 mg/mL), so that PCRP had an inhibitory effect on α-glucosidase, which was stronger than that of acarbose. In this study, the inhibitory IC_{50} value of the new acidic polysaccharide extracted from the Roxburgh rose fruit was 4.15 mg/mL, which was higher than the IC_{50} of PCRPs-1, indicating that PCRPs-1 had a stronger ability to inhibit α-glucosidase [23]. Wei Li et al. [11] reported that the IC_{50} value of PCRPs-1 inhibiting α-glucosidase was 2.19 mg/mL, which was higher than the IC_{50} of PCRPs-1.

Results and Analysis of the Antioxidant Activity of PCRP

Oxidation plays an important role in the production of energy to facilitate biological processes in many organisms. The accumulation of too many free radicals in the body can cause damage to DNA, proteins, and other biomolecules, leading to Alzheimer's disease, cardiovascular disease, diabetes, and other diseases [16]. Therefore, it is especially important to remove or inhibit free radicals. Numerous studies at home and abroad have shown that the flavonoids, polysaccharides, and volatile oils in PCR have antioxidant effects, but polysaccharides have been reported less.

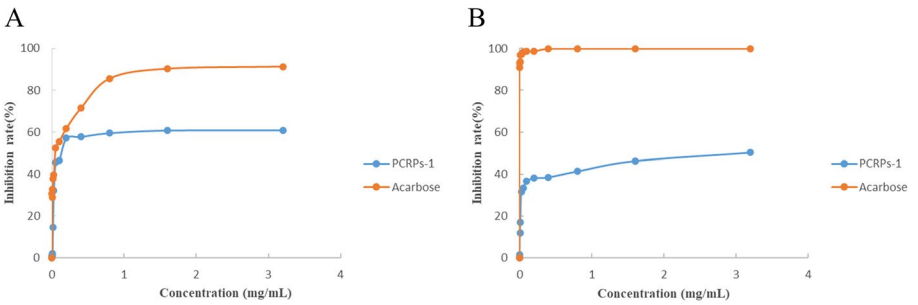

Fig. 5 The α-amylase inhibitory activities (**A**), α-glucosidase inhibitory activities (**B**) of PCRPs-1

DPPH Free Radical Scavenging Capacity

As shown in Fig. 6A, when the concentration of PCRPs-1 and VitC gradually increased, its scavenging effect on DPPH radicals gradually increased. After calculation, it was learned that the IC_{50} value of PCRPs-1 was 0.197 mg/mL, which was significantly higher than that of VitC (0.032 mg/mL), so PCRP had a scavenging effect on DPPH free radicals, but its effect was much weaker than that of VitC. In this study, the scavenging activity of water-soluble polysaccharides on DPPH free radicals was studied. After purification, the IC_{50} of polysaccharide on DPPH free radicals was 11.578 mg/mL, respectively, which was higher than that of PCRPS-1, indicating that PCRPS-1 had a strong scavenging effect on DPPH free radicals [9]. Wei Li et al. [12] measured the IC_{50} value of polysaccharides from *Platycodon grandiflorum* against DPPH· (1.561 mg/mL).

ABTS•$^{+}$ Free Radical Scavenging Capacity

As shown in Fig. 6B, when the concentration of PCRPs-1 and VitC gradually increased, its scavenging effect on ABTS•$^{+}$ free radicals increased. The IC_{50} of PCRPs-1 was 16.9 mg/mL, which was significantly higher than that of VitC (0.0014 mg/mL); therefore, PCRP had a scavenging effect on the scavenging of ABTS•$^{+}$ free radicals, but the effect was much weaker than that of VitC. Wei Li et al. [12] measured that the IC_{50} value of polysaccharides from *Platycodon grandiflorum* against ABTS·$^{+}$ (0.724 mg/mL).

Analysis of Results

The results of the PCRP derivatized samples and the monosaccharide control are shown in Fig. 7.

From Fig. 7, it can be seen that the peel polysaccharides consist of mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose, and arabinose with a molar ratio of 1: 39.24: 4.41: 8.91: 7.83: 86.00: 1.02: 9.17. Yapeng Li et al. [13] reported that the Pericarpium Citri Reticulatae "Chachiensis" polysaccharide extracted

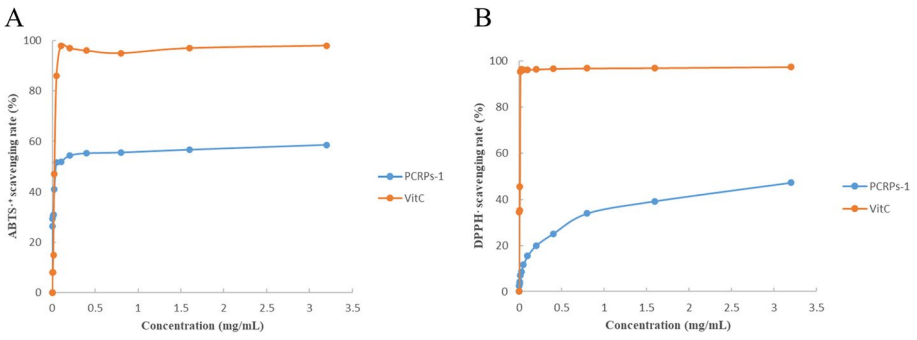

Fig. 6 The DPPH· scavenging activities (**A**), and ABTS·$^{+}$ scavenging activities (**B**) of PCRPs-1

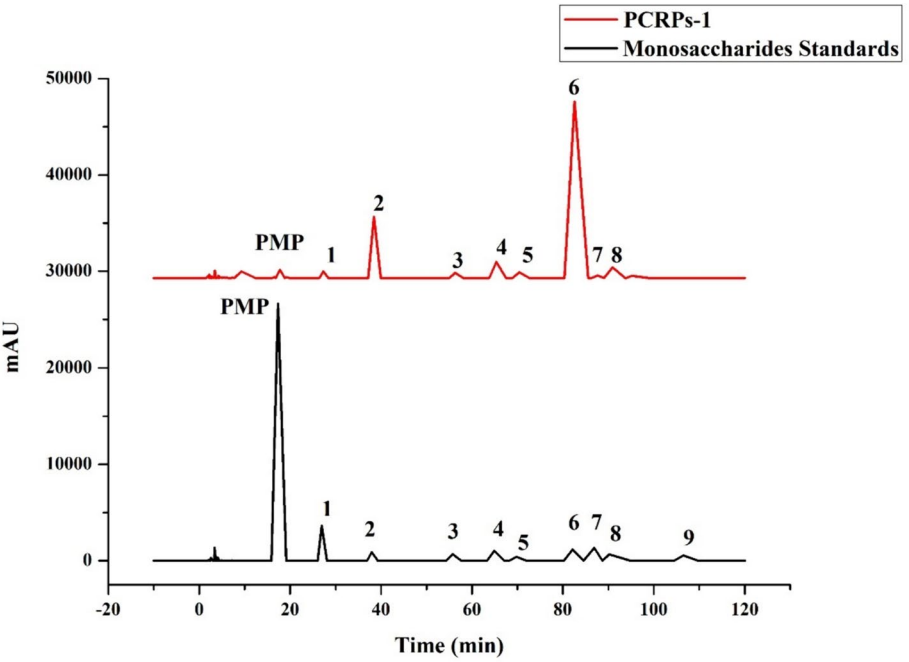

Fig. 7 HPLC of 9 monosaccharide standards and PCRPs-1

by ultrasonic assisted extraction were composed of D-galacturonic acid (51.17%), arabinose (25.63%), galactose (12.25%), and glucose (5.46%), which was different from the molar ratio of the results of this experiment.

Conclusion

In this thesis, ultrasound-assisted low eutectic solvent extraction of PCRP was carried out, and the results showed that with a material-liquid ratio of 1:37 g/mL, a water content of 44%, a time of 89 min, and a power of 320 W, the polysaccharide extraction rate was measured to be 5.41%, which is optimized compared with the extraction rate of 3.92% in the ordinary aqueous extraction method. By α-glucosidase inhibitory activity assay, the IC_{50} value of PCRPs-1 was 1.82 mg/mL, which was significantly lower than that of acarbose (3.42 mg/mL), indicating that PCRPs-1 had a strong inhibitory effect on α-glucosidase activity. The IC_{50} of PCRPs-1 was 2.84 mg/mL by α-amylase inhibitory activity assay. The IC_{50} value of PCRPs-1 was 0.197 mg/mL by DPPH free radical scavenging activity assay. The IC_{50} value of PCRPs-1 was 16.9 mg/mL by $ABTS\bullet^{+}$ free radical scavenging activity assay. Analyzing the monosaccharide fractions of PCRPs-1, it can be seen that PCRPs-1 consisted of mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose, arabinose, and their molar ratios were 1: 39.24: 4.41: 8.91: 7.83. 86.00: 1.02: 9.17. The structural analysis and the mechanism of hypoglycemic and antioxidant activities of PCRPs-1 need to be further investigated. It is hoped that more, in-depth and diverse reports on the preparation, structural analysis, and activity of PCRP and their derivatives will be available in the future.

Author Contribution Methodology, Y.W.; investigation, Y.W., and J.C.; formal analysis, Y.W., and J.C.; resources, Y.W.; writing—original draft preparation, J.C.; writing-review and editing, Y. W. and J.C.; supervision, Y.W.; project administration, Y.W.; funding acquisition, Y.W.

Funding This research work was financially supported by the Guangdong Basic and Applied Basic Research Foundation (No. 2021A1515110565) and the Science and Technology Program of Guangzhou, China (No. 202201010530).

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

Declarations

Ethical Approval This article contains no studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable. This study did not use any human or animal subjects.

Consent for Publication Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Competing Interests The authors declare no competing interests.

References

1. Bayram, S., Kutlu, N., Gerçek, Y. C., Çelik, S., & EcemBayram, N. (2022). Bioactive compounds of deep eutectic solvents extracts of Hypericum perforatum L.: Polyphenolic- organic acid profile by LC-MS/MS and pharmaceutical activity. *Food Bioscience, 49*, 101926.
2. Chen, R., Li, Y., Dong, H., Liu, Z., Li, S., Yang, S., & Li, X. (2012). Optimization of ultrasonic extraction process of polysaccharides from *Ornithogalum CaudatumAit* and evaluation of its biological activities. *Ultrasonics Sonochemistry, 19*, 1160–1168.
3. Chen, Z., Liu, Y., Wang, D., Wu, N., Wang, K., & Zhang, Y. (2022). Preparation, chemical structure and α-glucosidase inhibitory activity of sulfated polysaccharide from *Grifolafrondosa*. *Journal of Functional Foods, 98*, 105289.
4. Dai, J., Wu, Y., Chen, S.-W., Zhu, S., Yin, H.-P., Wang, M., & Tang, J. (2010). Sugar compositional determination of polysaccharides from *Dunaliellasalina* by modified RP-HPLC method of precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone. *Carbohydrate Polymers, 82*, 629–635.
5. Della Posta, S., Gallo, V., Gentili, A., & Fanali, C. (2022) Strategies for the recovery of bioactive molecules from deep eutectic solvents extracts. *TrAC Trends in Analytical Chemistry*, *116798*, 1–42.
6. Deng, Y., Huang, L., Zhang, C., Xie, P., Cheng, J., Wang, X., & Liu, L. (2020). Novel polysaccharide from *Chaenomelesspeciosa* seeds: Structural characterization, α-amylase and α-glucosidase inhibitory activity evaluation. *International Journal of Biological Macromolecules, 153*, 755–766.
7. Du, B., Peng, F., Xie, Y., Wang, H., Wu, J., Liu, C., & Yang, Y. (2022). Optimization Extraction and Antioxidant Activity of Crude Polysaccharide from Chestnut Mushroom (*Agrocybeaegerita*) by Accelerated Solvent Extraction Combined with Response Surface Methodology (ASE-RSM). *Molecules, 27*, 2380.
8. Hu, X., Xu, F., Li, J., Li, J., Mo, C., Zhao, M., & Wang, L. (2022). Ultrasonic-assisted extraction of polysaccharides from coix seeds: Optimization, purification, and in vitro digestibility. *Food Chemistry, 374*, 31636,1.
9. Jeddou, K. B., Chaari, F., Maktouf, S., Nouri-Ellouz, O., Helbert, C. B., & Ghorbel, R. E. (2016). Structural, functional, and antioxidant properties of water-soluble polysaccharides from potatoes peels. *Food Chemistry, 205*, 97–105.
10. Li, J., Chen, Z., Shi, H., Yu, J., Huang, G., & Huang, H. (2023). Ultrasound-assisted extraction and properties of polysaccharide from *Ginkgo biloba* leaves. *Ultrasonics Sonochemistry, 93*, 106295.
11. Li, W., Zhang, Y., Sang, L., Zhao, X., & Xie, J. (2023). Effects of different extraction techniques on the structural, physicochemical, and bioactivity properties of heteropolysaccharides from *Platycodon grandiflorum* roots. *Process Biochemistry, 127*, 33–43.

12. Li, W., Zhang, Y., Zhao, X., Fang, L., Yang, T., & Xie, J. (2023). Optimization of ultrasonic-assisted extraction of *Platycodon grandiflorum* polysaccharides and evaluation of its structural, antioxidant and hypoglycemic activity. *Ultrasonics Sonochemistry, 100*, 106635.
13. Li, Y., Li, Z., Chen, B., Hou, Y., Wen, Y., Gan, L., Jin, J., Li, C., Wu, P., Li, D., Chen, W. H., & Wu, R. (2023). Ultrasonic assisted extraction, characterization and gut microbiota-dependent anti-obesity effect of polysaccharide from *PericarpiumCitriReticulatae 'Chachiensis'*. *Ultrasonics Sonochemistry, 95*, 106383.
14. Li, Z.-R., Jia, R.-B., Wu, J., Lin, L., Ou, Z.-R., Liao, B., Zhang, L., Zhang, X., Song, G., & Zhao, M. (2021). *Sargassumfusiforme* polysaccharide partly replaces acarbose against type 2 diabetes in rats. *International Journal of Biological Macromolecules, 170*, 447–458.
15. Liu, Y. C., Tang, X. C., Hu, H. P., Chen, D. L., & Wu, Q. P. J. A. E. (2021). *Genetic diversity and main functional composition of Lingzhi strains from main producing areas in China, 11*, 1–13.
16. Liu, Z.-Q. (2020). Bridging free radical chemistry with drug discovery: A promising way for finding novel drugs efficiently. *European Journal of Medicinal Chemistry, 189*, 112020.
17. Meenu, M., Bansal, V., Rana, S., Sharma, N., Kumar, V., Arora, V., & Garg, M. (2023). Deep eutectic solvents (DESs) and natural deep eutectic solvents (NADESs): Designer solvents for green extraction of anthocyanin. *Sustainable Chemistry and Pharmacy, 34*, 101168.
18. Mohammed, J. K., Mahdi, A. A., Ahmed, M. I., Ma, M., & Wang, H. (2020). Preparation, deproteinization, characterization, and antioxidant activity of polysaccharide from *Medemiaargun* fruit. *International Journal of Biological Macromolecules, 155*, 919–926.
19. Oudjeriouat, N., Moreau, Y., Santimone, M., Svensson, B., Marchis-Mouren, G., & Desseaux, V. (2003). On the mechanism of α-amylase. *European Journal of Biochemistry, 270*, 3871–3879.
20. Saravana, P. S., Cho, Y.-N., Woo, H.-C., & Chun, B.-S. (2018). Green and efficient extraction of polysaccharides from brown seaweed by adding deep eutectic solvent in subcritical water hydrolysis. *Journal of Cleaner Production, 198*, 1474–1484.
21. Shang, X.-C., Chu, D., Zhang, J.-X., Zheng, Y.-F., & Li, Y. (2021). Microwave-assisted extraction, partial purification and biological activity in vitro of polysaccharides from bladder-wrack (*Fucus vesiculosus*) by using deep eutectic solvents. *Separation and Purification Technology, 259*, 1–8.
22. Tan, J., Cui, P., Ge, S., Cai, X., Li, Q., & Xue, H. (2022). Ultrasound assisted aqueous two-phase extraction of polysaccharides from *Cornusofficinalis* fruit: Modeling, optimization, purification, and characterization. *Ultrasonics Sonochemistry, 84*, 105966.
23. Wang, L., Chen, C., Zhang, B., Huang, Q., Fu, X., & Li, C. (2018). Structural characterization of a novel acidic polysaccharide from *Rosa roxburghii* Tratt fruit and its α-glucosidase inhibitory activity. *Food & Function, 9*, 3974–3985.
24. Wang, N., & Li, Q. (2022). Study on extraction and antioxidant activity of polysaccharides from *Radix Bupleuri* by natural deep eutectic solvents combined with ultrasound-assisted enzymolysis. *Sustainable Chemistry and Pharmacy, 30*, 100877.
25. Wu, D.-T., Feng, K.-L., Huang, L., Gan, R.-Y., Hu, Y.-C., & Zou, L. (2021). Deep eutectic solvent-assisted extraction, partially structural characterization, and bioactivities of acidic polysaccharides from lotus leaves. *Foods, 10*, 2330.
26. Yao, J., Zeng, J., Tang, H., Cheng, Y., Tan, J., Li, T., Li, X., He, J., & Zhang, Y. (2023). Effect of deep eutectic solvent extraction on *Auriculariaauricula* polysaccharide solubilization and antioxidant potential. *Sustainable Chemistry and Pharmacy, 34*, 101166.
27. Yingrui, W., Zheng, L., Guoyan, L., & Hongjie, W. (2022). Research progress of active ingredients of *Scutellariabaicalensis* in the treatment of type 2 diabetes and its complications. *Biomedicine & Pharmacotherapy, 148*, 112690.
28. Yu, X., Sun, S., Guo, Y., Liu, Y., Yang, D., Li, G., & Lü, S. (2018). *CitriReticulataePericarpium* (Chenpi): Botany, ethnopharmacology, phytochemistry, and pharmacology of a frequently used traditional Chinese medicine. *Journal of Ethnopharmacology, 220*, 265–282.
29. Yue, F., Zhang, J., Xu, J., Niu, T., Lü, X., & Liu, M. (2022). Effects of monosaccharide composition on quantitative analysis of total sugar content by phenol-sulfuric acid method. *Frontiers in Nutrition, 9*, 963318.
30. Yun, C., Ji, X., Chen, Y., Zhao, Z., Gao, Y., Gu, L., She, D., Ri, I., Wang, W., & Wang, H. (2023). Ultrasound-assisted enzymatic extraction of *Scutellariabaicalensis* root polysaccharide and its hypoglycemic and immunomodulatory activities. *International Journal of Biological Macromolecules, 227*, 134–145.
31. Zhang, L., & Wang, M. (2017). Optimization of deep eutectic solvent-based ultrasound-assisted extraction of polysaccharides from *Dioscoreaopposita* Thunb. *International Journal of Biological Macromolecules, 95*, 675–681.
32. Zhang, Y., He, L., Li, Q., Cheng, J., Wang, Y., Zhao, J., Yuan, S., Chen, Y., & Shi, R. (2022). Optimization of ultrasonic-assisted deep eutectic solvent for the extraction of polysaccharides from

Indocalamus tessellatus leaves and their biological studies. *Sustainable Chemistry and Pharmacy, 30*, 100855.
33. Zhao, T., Li, C., Wang, S., & Song, X. (2022). Green Tea (*Camellia sinensis*): A Review of Its Phytochemistry, Pharmacology, and Toxicology. *Molecules, 27*, 3909.
34. Zhu, H., Tian, L., Zhang, L., Bi, J., Song, Q., Yang, H., & Qiao, J. (2018). Preparation, characterization and antioxidant activity of polysaccharide from spent *Lentinusedodes* substrate. *International Journal of Biological Macromolecules, 112*, 976–984.