American Journal of Biochemistry and Biotechnology

Original Research Paper

Evaluation of Antioxidant and Antibacterial Activities of the Polysaccharides from Mycelium and the Culture Medium of *Pleurotus Eryngii* Strain UN-1

Qing Liu, Haodong Wu, Ying Wu, Mengyu Liu, Yao Zhang and Yuanda Song

Department of Food Science and Technology, Shandong University of Technology, China

Article history
Received: 25-04-2022
Revised: 20-06-2022
Accepted: 23-06-2022

Corresponding Author:
Qing Liu
Department of Food Science and Technology, Shandong University of Technology, China
Email: qingliu0906@sdut.edu.cn

Abstract: *Pleurotus eryngii* was one of the most precious and ordinary edible mushrooms, and its polysaccharides were the major active substances. In this study, *P. eryngii* strain UN-1 was cultured in a liquid medium at 25°C under a shaking of 80 rpm for 7 days. Two crude polysaccharides named CPN and CPNM were obtained from the mycelium of *P. eryngii* UN-1 and its broth culture medium, respectively. After partial purification by cellulose DEAE-52 column chromatography and Sephadex G-100 gel-filtration, two fractions (PNP-1 and PNP-2) from CPN and one (PNMP-1) from CPNM were eluted and subjected to physicochemical properties, antioxidant, and antibacterial activities, in addition, the crude polysaccharides. The *in vitro* antioxidant assay revealed that all samples showed moderate scavenging capacities on DPPH radical (6.31-80.92%), ABTS radical (9.33-67.26%), and hydroxyl radical (11.21-79.53%) at different concentrations (0-2000 μg/mL), and PNP-2 exhibited the highest scavenging activity for all tested free radicals. Moreover, the antibacterial activity of the most active fraction (PNP-2) showed a significant effect against Escherichia coli, Bacillus Subtillis, Salmonella enterica, and Staphylococcus aureus subsp. Aureus strains with Minimum Inhibitory Concentration (MIC) values (2.5, 5, 2.5 and 5 mg/mL, respectively). Notably, the UV-vis Spectrophotometer confirmed that multiple active known compounds such as uremic acid were detected in all tested fractions, which confirmed the antioxidant and antimicrobial activities obtained in this study. Conclusively, the polysaccharides obtained from *P. eryngii* UN-1 have the potential to act as a functional food ingredient or a preventive drug against human pathogenic bacteria.

Keywords: *Pleurotus Eryngii*, Polysaccharide, Purification, Antioxidant Activity, Antibacterial Activity

Introduction

In the last few decades, due to the pollution of the environment, accelerated growth of the human population, and stressful modern lifestyle, more and more consumers have become increasingly conscious of the necessity of a safe and nutritious diet. Mushrooms have become a significant source of nutraceuticals in solving these problems and meeting the requirements to help humans maintain health. In recent years, various edible mushrooms have become extremely popular as a dietary functional food owing to their good taste and high nutritional value. *Pleurotus eryngii*, (Family: Pleurotaceae, Class: Agaricomycetes, Order: Agaricales, Genus: *Pleurotus*) known as the Bailingu oyster mushroom in China, is commonly grown and cultivated in Europe, the Middle East, North America and many areas in Asia (Kim *et al*., 2013; Liu *et al*., 2013). It has attracted considerable attention because rich in protein, carbohydrates, unsaturated fatty acids, vitamins, and other nutrients (Zhang *et al*., 2020).

In recent years, antibacterial and antioxidants are two important aspects and trends to ensure food safety and health care functions. A large number of researches focus on extracting natural active ingredients from nature for the development of healthy food and medicine for the treatment of corresponding chronic diseases (Chzhu *et al*., 2020; Ismy *et al*., 2022; Lae *et al*., 2019).

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Polysaccharide, a high molecular weight polymer, consists of more than ten monosaccharides mutually joined by glycosidic linkages. Presently, the polysaccharides of mushroom origin have been widely studied and found to be important functional biological macromolecules due to their significant benefit to human health such as antioxidant, antidiabetic, immune potentiation, antitumor, anti-inflammatory, and hypoglycemic effects (Cheng *et al*., 2018; Rathore *et al*., 2019; Ruiz-Herrera and Ortiz-Castellanos, 2019). They have been widely applied to the food and drug industry because they are sourced naturally and have fewer side effects than synthetic ones. Currently, most of the research reports have focused on the polysaccharides extracted from fruiting bodies of *P. eryngii*, which have proved to be potential sources of natural immunomodulatory (Ren *et al*., 2016), antitumor (Ren *et al*., 2017), hepato-cardioprotective (Xu *et al*., 2016) and neuroprotective compounds (Zhang *et al*., 2018).

Zhang *et al*. (2016a). have extracted and purified two polysaccharide fractions (IPS-1 and IPS-2) from *P. eryngii* SI-04 mycelia and IPS-2 had significant hepatoprotective effects and ameliorating the hepatic structure damage. Ma *et al*. (2016) used response surface methodology to optimize the extraction of polysaccharides from *P. eryngii*. The crude polysaccharide was purified and the two fractions (CPPS-1 and CPPS-2) were obtained. The antioxidant and cytotoxicity analysis showed that CPPS-1 had the strongest activity. Xu *et al*. (2016) have purified a water-soluble Polysaccharide (EPA-1) from *P. eryngii* and EPA-1 and possessed a good immune regulatory activity *in vivo*. Many types of research have focused on the mycelial polysaccharide from *P. eryngii* because of its short growth cycle, low cost, high yield, and healthcare function. However, the polysaccharides from the fermentation broth were rarely studied, as well as the comparison of the bioactivities of both intracellular and extracellular polysaccharides from *P. eryngii*.

In the current study, as shown in Fig. 1, we cultured the *P. eryngii* UN-1 with a liquid suspension method, extracted and purified the crude polysaccharides with DEAE-52 cellulose column, and Sephadex G-100 column from mycelium of *P. eryngii* UN-1 and its suspension culture medium, respectively. Then, their physicochemical properties, antioxidant and antibacterial activities were further investigated *in vitro*.

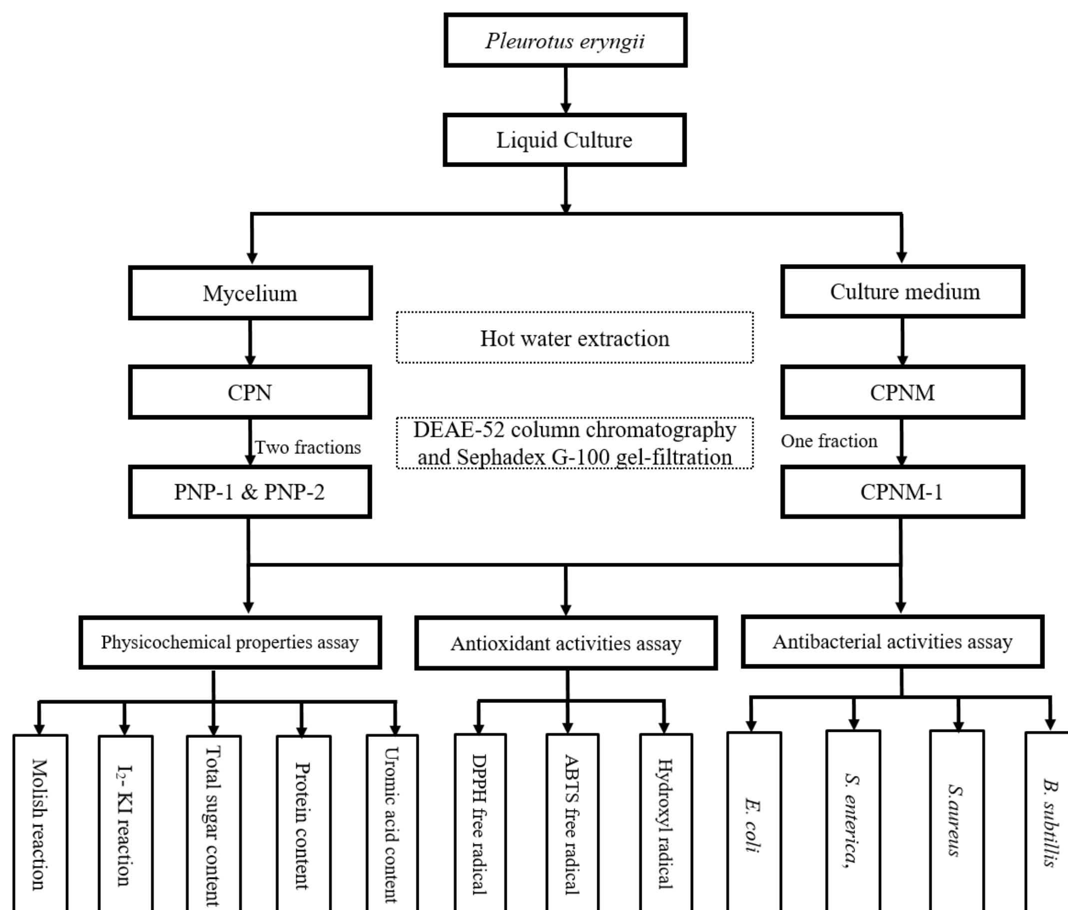

Fig. 1: Should be cited here

Materials and Methods

Materials and Reagents

Trichloroacetic Acid (TCA), 1,1-Diphenyl-2-Picrylhydrazyl (DPPH), 2, 2'-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS), Folin-Ciocalteu and Ascorbic acid were purchased from Sigma Aldrich Chemical Co. (St. Louis, USA). Cellulose DEAE-52 and Sephadex G-100 columns were purchased from the Solaria Science and Technology Co., Ltd. (Beijing, China). All other chemicals and reagents used in this study were of analytical grade.

Strains and Fermentation

The *P. eryngii* UN-1 strain used in this experiment was isolated and maintained in our lab. It was initially cultivated on Potato Dextrose Agar (PDA) medium in Petri dishes under 25°C for 6 days. Excise ten 5-mm^2 agar-plate cultures using a sterile puncher and inoculate into a 500 mL flask containing 250 mL basal liquid medium. Each liquid medium containing potato (180 g/L), glucose (25 g/L), KH_2PO_4 (1.6 g/L), yeast extract powder (10 g/L) and $MgSO_4 \cdot 7H_2O$ (0.8 g/L). The liquid broth was subjected to sterilization at 121°C for 15 min and was kept cultivation at 25°C under a shaking of 80 r/min for 7 days in the dark.

Extraction of Polysaccharides

The polysaccharides were extracted according to the protocol described by Jiang *et al.* (2015) with some modifications. The culture *P. eryngii* UN-1 mycelia were separated by centrifugation at 4000 r/min for 15 min using a 5810R centrifugator (Eppendorf, Germany). Then the mycelia were collected, washed with distilled water, and lyophilized with a Labconco Free Zone®Triad™ (MO, USA). The freeze-dried fresh mycelia were ground using an FSJ-1000A high-speed pulverizer (Guangzhou Xinjiate, China) and sifted with 100 mesh. The mycelia power of *P. eryngii* UN-1 was extracted twice with distilled water (1:50, w/v) at 90°C for 6 h. All the aqueous extracts were combined and concentrated using a RE-52AA rotary evaporator (Shanghai Yarong, China) to an appropriate volume and 80% (w/v) TCA was added to remove protein. The mixture was left at 4°C for 6 h. After centrifugation at 5000 rpm for 15 min, the supernatant was collected, concentrated using a rotary evaporator, and pelleted by adding ethanol (4-fold volumes) overnight at 4°C. The precipitation was collected and lyophilized to obtain the Crude mycelia Polysaccharides (CPN). The medium supernatant of liquid fermentation was collected, concentrated, deproteinized, and precipitated with 4-fold volume ethanol for 24 h at 4°C. After centrifugation at 4000 rpm for 10 min, the Crude medium Polysaccharides (CPNM) were obtained for further processing.

Purification of Polysaccharides

The above CPN and CPNM were firstly applied to a DEAE-52 cellulose column (2.6 × 40 cm). A stepwise gradient of NaCl (0, 0.1, 0.2, 0.3 and 0.4 mol/L) with a flow rate of 1.0 mL/min was used to elute out the subfractions. The eluate was collected at10 mL/tube using an automatic collector. The presence of polysaccharides in all the fractions was assayed spectrophotometrically by a sulfuric acid-phenol method using a SHIMADZU UV-2600 ultraviolet spectrophotometer (Tianjin, China). The major eluate was collected separately and further infused into a Sephadex G-100 size-exclusion column (1.3 × 50 cm) via chromatographic elution with distilled water at a flow rate of 0.3 mL/min. The main fractions were pooled, concentrated, and lyophilized for further analysis.

Based on the purification profile, two fractions were identified in CPN and one in CPNM. The concentrates of the three eluates were dialyzed and lyophilized to give purified polysaccharides, named PNP-1, PNP-2, and PNMP-1, respectively.

Molish Reaction

The Molish reaction was performed based on a previous report (Liu *et al.*, 2018). The polysaccharide solution (1 mg/mL, 200 μL) was mixed with Molish reagent (α-naphthol ethanol solution, 2%, 150 μL). Then 500 μL of sulfuric acid was added carefully. Distilled water was used as a negative contrast, while a 0.5% starch indicator was used as a positive contrast. The color change of the interface between sulfuric acid and the sugar solution was assayed.

I_2-KI Reaction

2 mL of polysaccharide solutions (1 mg/mL) was mixed with 1.0 mL of 0.2% KI solution. The mixture was shaken thoroughly and the color change was observed. Distilled water was used as a negative contrast, while a 0.5% starch indicator was a positive contrast.

Chemical Analysis

The sugar content of samples was measured following the phenol-sulfuric acid colorimetric method (Dubois *et al.*, 1956). Protein content was determined using Braford's method (Bradford, 1976). The uremic acid content was assayed by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973).

Assay of DPPH Radical Scavenging Activity in Vitro

Radical scavenging activity was carried out according to a method mentioned by (Liu *et al.*, 2018). 2 mL of aqueous solutions of CPN, CPNM, and purified fractions with different concentrations were mixed with 2 mL of 0.2 mmol/L DPPH solution. The reaction mixture was vigorously shaken and left to stand at room temperature in the dark for 30 min. An optical density of 517 nm was

used for the measurement of DPPH radical absorption. Ascorbic acid was used as a positive contrast. The control sample was prepared by replacing the DPPH solution with ethanol and deionized water was used instead of the polysaccharide sample solutions to give a blank sample. The result was calculated based on the following equation:

$$DPPH\ radical\ scavenging\ activity(\%)$$
$$=1-\frac{A_{sample}-A_{control}}{A_{blank}}\times 100\%$$

Assay of ABTS Radical Scavenging Activity in Vitro

The ability of polysaccharides to scavenge ABTS radical was measured based on previous literature (Wang *et al*., 2010), with slight modifications. ABTS radical cation solution was prepared by diluting a 7 mmol/L ABTS stock solution at a ratio of 1:1 (v/v) with potassium persulfate (2.4 mm) and incubating it at 20°C for 16 h in the dark. Phosphate buffered saline (pH 7.4) was used to adjust the ABTS radical solution to have an absorbance between 0.75-0.8 under 734 nm. Then mix 180 μL of ABTS reagent with 20 μL of each test sample. The scavenging ability of the fractions was compared with that of ascorbic acid. The blank sample was prepared with the ABTS free radical solution instead of the polysaccharide sample solutions. To evaluate the ability of polysaccharides to scavenge ABTS free radicals, the formula is as follows:

$$ABTS\ radical\ scavenging\ activity\ (\%)=$$
$$=\frac{A_{blank}-A_{sample}}{A_{blank}}\times 100\%$$

Assay of Hydroxyl Radical Scavenging Activity in Vitro

The hydroxyl radical scavenging activity of polysaccharides was measured using the salicylic acid method referring to a previously published method with some modification (Wu *et al*., 2020). Incubate a mixture of 1 mL polysaccharide solution with different concentrations, 1 mL ferrous sulfate solution (1.5 mmol/L), and 1 mL of ethanol salicylic acid solution in test tubes. After shaking and standing for 10 min, add 1 mL of H_2O_2 (2.4 mmol/L). The tube was left in dark for 30 min under 37°C, then measure the absorbance at 517 nm. Salicylic acid in ethanol was used to prepare the control sample and the blank sample was made by replacing the sample solution with deionized water. Ascorbic acid was used as a positive contrast. The result was calculated based on the following formula:

$$Hydroxyl\ radical\ scavenging\ activity\ (\%)\ =$$
$$=1-\frac{A_{sample}-A_{control}}{A_{blank}}\times 100\%$$

Anti-Microbiological Activity Assay

The antibacterial activity of polysaccharides was evaluated against four pathogenic bacteria including *Escherichia coli* (*E. coli*) CMCC 44102, *Bacillus Subtillis* (*B. subtillis*) CCTCCAB206681, *Salmonella enterica* (*S. enterica*) BNCC132287 and *Staphylococcus aureus subsp. aureus* (*S.aureus*) CMCC26003, which was purchased from BeNa Culture Collection (Beijing, China). The CPN, PNP-1, PNP-2, CPNM and PNMP-1 were diluted with sterilized 0.85% NaCl to 10 mg/mL. Each bacterium was activated and the final dose rate was optimized to 10^5 cfu/mL using sterile peptone water. In the next step, the overnight grown cultures were distributed by pouring 15-20 mL of pre-sterilized media onto the plate. After solidification, various holes of 7.8 mm diameter were created on the agar surface with a sterile stainless-steel cylinder. 100 μL of polysaccharide sample was loaded into each well for all the test bacteria and incubated at 37°C for 24 h. Eventually, the agar disc diffusion method by measuring and recording the diameter of inhibitory zones formed around the wells was used to assess the antimicrobial capacity. Penicillin solution (1 mg/mL) and NaCl (0.85%), were used as positive and negative controls, respectively.

Minimum Inhibitory Concentration (MIC) Determination

The MIC of all samples was detected by the double broth dilution method according to Wang *et al*. (2019) with some modifications. 100 μL of CPN, PNP-1, PNP-2, CPNM and PNMP-1 samples with series of concentrations (10.0, 5.0, 2.5, 1.25 and 0.625 mg/mL) were added into a 96-well plates. Then, 100 μL of bacterial suspension of 106 CFU/mL was loaded directly into the 96-well plates. After incubation under 37°C for 24 h, the lowest concentration of polysaccharides inhibiting bacteria growth was recorded as the MIC. The microplate with 200 μL 0.85% NaCl saline was used as the contrast.

Statistical Analysis

Significance analysis and one-way analysis of ANOVA by Duncan's test were conducted with SPSS 22.0 software. Each analysis was done in triplicate. The results were presented as the means ± Standard Deviation (SD) and $p<0.05$ was considered statistically significant.

Results and Discussion

Isolation and Purification of Polysaccharides

Figure 2 the elution profile of CPN (A) and CPNM (B) on DEAE-52 cellulose column eluted with distilled water and different stepwise gradient of NaCl aqueous solutions (0.1, 0.2, 0.3, and 0.4 mol/L).

Figure 3 the elution profile of PNP-1 (A), PNP-2 (B) and PNMP-1 (C) on Sephadex G-100 chromatography eluted with distilled water.

Crude polysaccharides CPN and CPNM were obtained from *P. eryngii* UN-1 mycelia and culture medium, respectively. The CPN was extracted from dry mycelia sample using the classical method of hot water extraction and CPNM was precipitated by ethanol from the deproteinized aqueous culture medium. The crude polysaccharides were subjected to a DEAE-52 cellulose column chromatography. The elution curve of the crude polysaccharides CPN and CPNM was presented in Fig. 2A and B. Two fractions, denoted as PNP-1and PNP-2, were collected from CPN eluted with 0.0 and 0.3 mol/L NaCl solutions. One fraction named PNMP-1was eluted with distilled water from CPNM. After concentrated and lyophilized, PNP-1, PNP-2, and PNMP-1 were further purified with a Sephadex G-100 column to yield three main final fractions. As illustrated in Fig. 3A, B, and C, the elution profiles showed three individual peaks, demonstrating the homogenization of the three fractions.

Table 1 physical and chemical properties of polysaccharides from *P. eryngii* NU-1.

Physical and Chemical Properties

The physicochemical properties of the polysaccharides are summarized in Table 1. The positive results of the Molish reaction found in all samples confirmed that the crude polysaccharides and fractions had the general properties of polysaccharides. Additionally, the I_2, KI assay did not provide any positive results indicating no starch in the samples. The total sugar contents analyzed in CPN and CPNM were 77.24 and 46.52%, respectively. After purification, the total carbohydrate of the two fractions extracted from mycelium reached 92.13% (PNP-1) and 94.96% (PNP-2). Similarly, the PNMP-1 was composed of 90.45% total sugar, which is significantly higher than the crude sample obtained from the culture medium (CPNM). All purified fractions contained low protein levels, indicating that this separation and purification procedure can effectively remove proteins. The uremic acid contents in PNP-1, PNP-2, and PNMP-1 were found to be 0.82, 10.35, and 0.51% in an m-hydroxydiphenyl analysis, respectively.

Figure 4 Antioxidant activities of the extracted polysaccharides. Scavenging effects of DPPH radicals (A); ABTS radicals (B); hydroxyl radicals (C).

DPPH Radical Scavenging Activity

Determination of the free radical scavenging ability of DPPH has been well used to assess the antioxidant activity of active substances. DPPH is an N-centered free radial that has been widely applied to research utilizing the free-radical scavenging ability or hydrogen donating capacity of compounds and pharmaceutical materials (Jiang *et al*., 2015). As shown in Fig. 4 A, the scavenging rate of polysaccharides on DPPH radicals. Notably, all polysaccharides possessed increased antioxidant activities significantly with the increasing concentration of samples from 0 to 2000 μg/mL. At a concentration of 2000 μg/mL, the DPPH radical scavenging rates of PNP-2, CPN, CPNM, PNP-1, and PNMP-1 were 80.92, 73.91, 62.83, 46.76, and 38.46%, respectively. The scavenging ability of PNP-2 was significantly higher than that of other polysaccharides ($P<0.05$), indicating that PNP-2 exhibited the strongest scavenging activity on the DPPH radical. CPN was found to have the second-highest scavenging activity and which is probably due to the complex composition of the crude polysaccharide. IPS-2 separated from *P. eryngii* SI-04 was reported to exhibit a DPPH free radical scavenging rate of 66.09% at a concentration of 1000 mg/L (Zhang *et al*., 2016b). PPM purified from *Phellinus pini* mycelia exhibited a strong DPPH scavenging activity (20 to 85%) at a concentration of 1-5 mg/mL (Jiang *et al.*, 2015). A study by Ma *et al*. (2016) also revealed that the CPPS-1 fraction extracted from *P. eryngii* at a concentration of 4000 μg/mL had a DPPH free radical scavenging effect of 65.60% (Ma *et al*., 2016). According to these results, it's indicated that the PNP-2 has an acceptable DPPH radical scavenging capacity.

ABTS Radical Scavenging Activity

The ABTS action radical is composed of ABTS, peroxide, and hydroperoxide. The measurement of ABTS radical scavenging activity was based on the decrease of absorbance at 734 nm when antioxidant compounds were added to inactivate the ABTS radicals (Liu *et al.*, 2019). The scavenging effect of polysaccharides on ABTS radical is shown in Fig. 4B, all the five samples expressed a scavenging effect in a concentration-dependent manner. At a concentration of 2000 μg/mL, PNP-2 showed the highest scavenging activity (67.26%). Yet the scavenging ability of PNMP-1 was 42.13%, which is much lower than that of ascorbic acid (96.52%). In addition, the ABTS radical scavenging activities of all samples followed the order: PNP-2>CPN>CPNM>PNP-1>PNMP-1. Their scavenging abilities are positively correlated with their uremic acid content. This finding is consistent with Liu's finding that the ABTS scavenging capacity of purified polysaccharides from *Helicteres Angustifolia* L. increased with the increase of their uremic acid (Liu *et al*., 2018).

Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are strong oxidative free radicals produced in the body, which can easily pass through the cell membrane and react with various macromolecules, leading to cell apoptosis and disintegration (Luo *et al.*, 2010). As illustrated in Fig. 4C, the hydroxyl radical scavenging activity of all the five samples was remarkable and dose-dependent in the test dosage range. At a concentration of 2000 μg/mL, scavenging rates of PNP-2, CPN, CPNM, PNP-1 and PNMP-1 were 79.53, 76.95, 67.15, 60.12 and 58.73%, respectively. However, the

scavenging rates of all samples were lower than that of ascorbic acid. According to Zhang's work, at a concentration of 1500 mg/L, a polysaccharide (MZPS-3) purified from *P. djamor* showed a hydroxyl radical scavenging rate of 69.40% (Zhang *et al.*, 2016a) and 79.27% of hydroxyl radical scavenging capacity were reported to be inhibited by PPM polysaccharide at 12.5 mg/mL from *Phellinus pini* (Jiang *et al.*, 2015). In general, compared with the polysaccharides obtained from broth Medium (CPNM and PNMP-1), the fractions from mycelia (CPN, PNP-1, and PNP-2) exhibited higher antioxidant activity within the measured concentration range from 0 to 2000 μg/mL at all free radical scavenging assay. That may be due to the ratio of monosaccharides and the difference in molecular weight between them (Jiang *et al.*, 2015). The above results show that the PNP-2 can be used as an effective antioxidant in food or pharmacy as an additive in the immediate future.

Table 2 antibacterial activity of polysaccharides and penicillin.

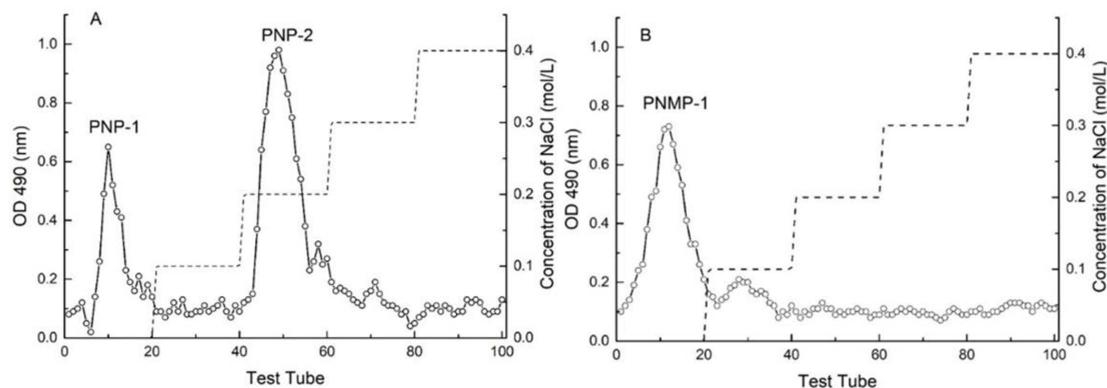

Fig. 2: Should be cited here

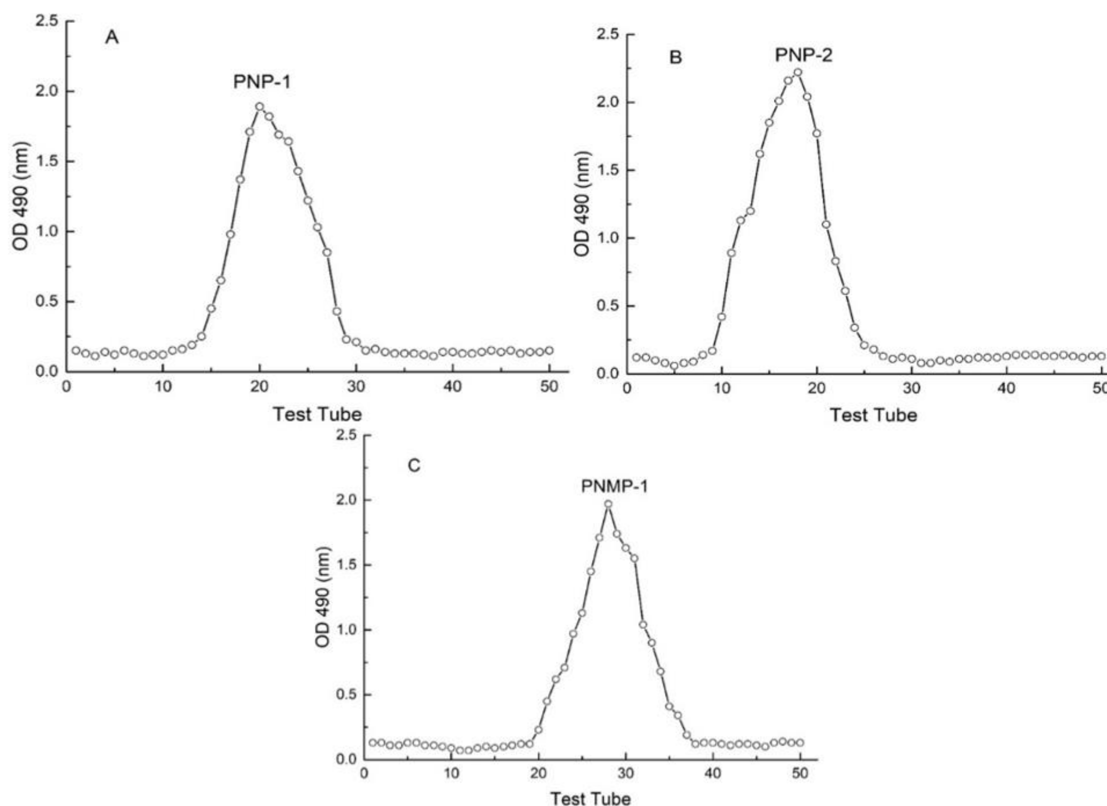

Fig. 3: Should be cited here

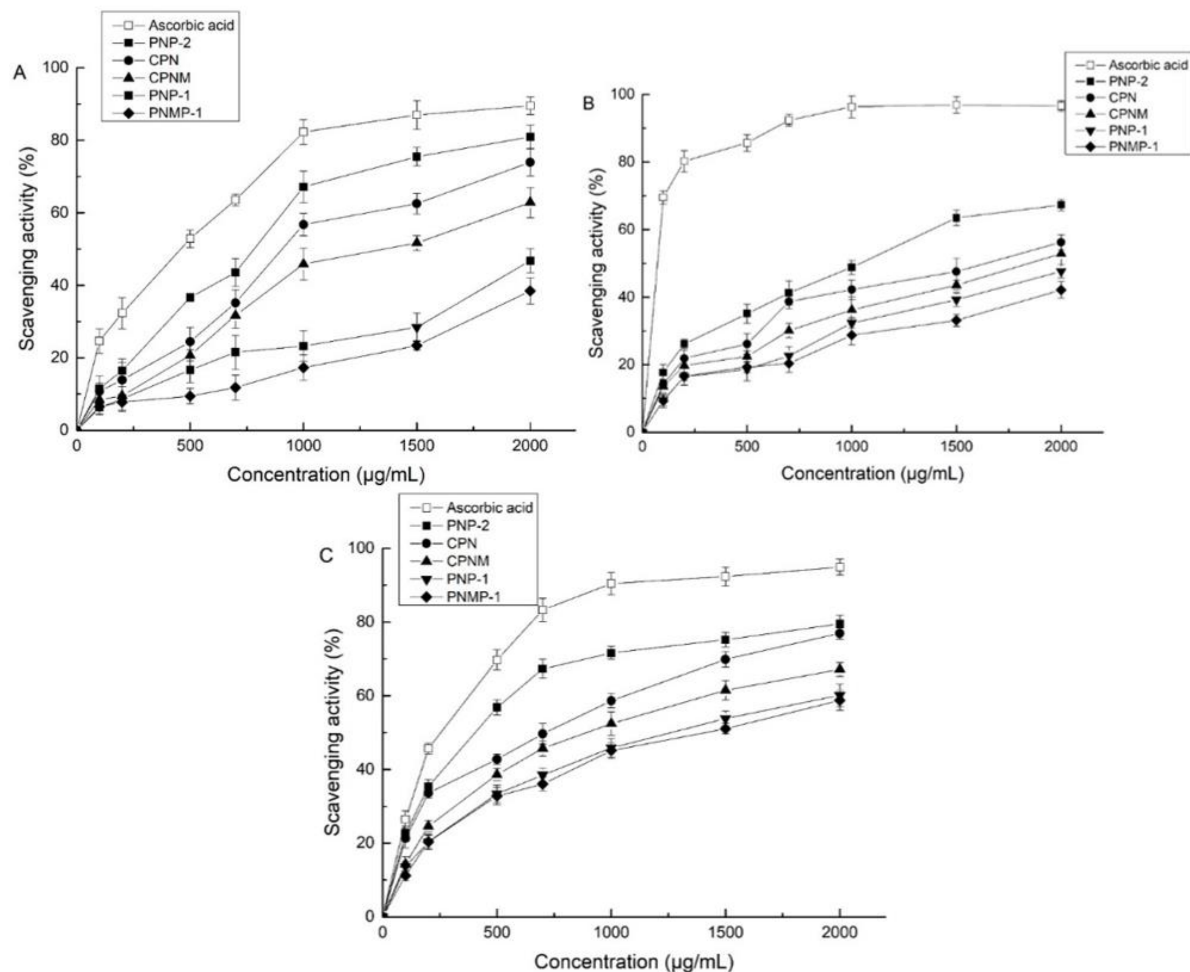

Fig. 4: Should be cited here

Table 1: Should be cited here

Sample	Molish reaction	KI reaction	Total sugars (%)	Protein (%)	Uremic acid (%)
CPN	Positive	Negative	77.24±4.33	18.22±2.07	7.73±1.27
PNP-1	Positive	Negative	92.13±6.91	0.41±0.07	0.82±0.02
PNP-2	Positive	Negative	94.96±6.64	0.37±0.03	10.35±0.75
CPNM	Positive	Negative	46.52±3.19	6.59±0.82	1.16±0.26
PNMP-1	Positive	Negative	90.45±5.88	0.62±0.09	0.51±0.03

Table 2: Should be cited here

	Diameters of inhibition zone (mm)*					
Spoilage bacteria	CPN	PNP-1	PNP-2	CPNM	PNMP-1	Penicillin
Gram negative						
E. coli	10.7±0.4[b]	9.3±0.3[b]	11.8±0.3[b]	9.9±0.4[b]	9.7±0.5[a]	16.2±0.4[b]
S. enterica	8.5±0.3[c]	8.1±0.2[c]	8.6±0.4[d]	8.1±0.2[c]	8.0±0.3[b]	14.7±0.4[c]
Gram positive						
S. aureus	13.2±0.6[a]	10.1±0.3[a]	14.1±0.7[a]	10.8±0.3[a]	9.7±0.2[a]	32.1±0.2[a]
B. subtillis	8.3±0.2[c]	8.2±0.2[c]	9.1±0.5[c]	8.2±0.3[c]	8.1±0.2[b]	10.9±0.3[d]

Values with no letters in common within each column are significantly different (p<0.05)
* Values are expressed as mean ± SD (n = 3)

Antibacterial Activity Assay

The antibacterial activity of all fractions (CPN, PNP-1, PNP-2, CPNM, and PNMP-1), was carried out by agar disc diffusion method against *E. coli*, *S. enterica*, *S. aureus*, and *B. subtillis* strains. As shown in Table 2, the results showed that all tested fractions displayed inhibitory effects against the four harmful bacteria. The four kinds of polysaccharides showed the biggest sensitivity to the *S. aureus* according to the diameters of inhibition zones. For the tested polysaccharides (CPN, PNP-1, PNP-2, CPNM, and PNMP-1) and penicillin, the diameters of the inhibition zones against *S. aureus* were 13.2±0.6, 10.1±0.3, 14.1±0.7, 10.8±0.3, 9.7±0.2 and 32.1±0.2 mm, respectively. Zhu *et al.* (2012). have extracted and purified two polysaccharides (PL1 and PL2) from a Spent Mushroom Substrate (SMS) and subjected them to antibacterial activities assay using *E. coli*, *S. aureus,* and *Sarcina lutea*. The antibacterial activity of the polysaccharide from SMS against *E. coli* was the highest, while the lowest was against *S. lutea*. It has been reported that the polysaccharide PEPS, extracted from *P. eryngii*, also showed a certain inhibiting activity on *E. coli* and *S. aureus* with inhibition zones of 10.1±0.2 mm and 17.2±0.8 mm at the concentration of 10 mg/mL (Li *et al.*, 2014). P3a (10 mg/mL), a polysaccharide isolated and purified from *P. eous*, exhibited a good and stable antibacterial effect with inhibition zones against *E. coli*, *Klebsiella pneumonia*, *S. aureus,* and *B. subtilis* with inhibition zones ranging from 8.1 to 14.2 mm (Gunasekaran *et al.*, 2021). These data indicated the polysaccharides from *Pleurotus spp.,* had a wide range of biological activity.

Meanwhile, the lowest concentrations of polysaccharides that prevented the four harmful bacteria were investigated. Our results of MIC were consistent with what has been observed in the inhibition zone measurement. All the aqueous samples were found to be most effective against *S. aureus*. Both PNP-2 and CPN exhibited the lowest MIC of 2.5 mg/mL against *S. aureus*, while PNP-1, CPNM, and PNMP-1 had the same MIC for *E. coli* (5 mg/mL), *S. enterica* (5 mg/mL) and *B. subtillis* (10 mg/mL), respectively. It can be observed that all the polysaccharides have antibacterial constituents and PNP-2 shows a stronger antimicrobial effect than other samples. A polysaccharide separated from *Cordyceps cicadae* has been reported to have a strong antibacterial activity against *E. coli*, *S. aureus*, *B. subtilis*, *Salmonella paratyphi*, and *Pseudomonas aeruginosa* and the MIC to *E. coli* was 0.10 mg/mL (Zhang *et al.*, 2017). It has been widely believed that the structure of polysaccharides is closely related to the antibacterial activity of polysaccharides of natural origin (Jiang *et al.*, 2020). And it has been demonstrated that uremic acid content is also an essential factor that influences the antibacterial activity of polysaccharides (Jiang *et al.*, 2020). The present work provided a scientific basis for the further study of the polysaccharides from *P. eryngii* UN-1.

Conclusion

In our study, three polysaccharides were isolated and purified from the mycelium (PNP-1 and PNP-2) and the culture broth (PNMP-1) of *P. eryngii* UN-1 using modern chromatographic tools. Based on the physicochemical properties, all the fractions possessed the polysaccharide general properties. The uremic acid concentration (10.35%) of PNP-2 was found to be higher than that of other fractions. The *in vitro* antioxidant assay found that all samples showed moderate scavenging capacities on DPPH radical, ABTS radical, and hydroxyl radical. PNP-2 has the highest scavenging activity of all antioxidant activity assays than other crude polysaccharides and fractions. The antibacterial activity of the PNP-2 fraction showed a significant effect against tested pathogenic strains with considerable MICs, whereas the other two fractions (PNP-1 and PNMP-1) were slightly effective. Our results suggest that the content of uremic acid might play a key role in their antioxidant and antibacterial activities. In addition, the polysaccharides obtained in this study could be suggested to be used in functional foods, cosmetics industries, and biological medicine due to their natural origin, low toxicity, strong biological activity, and fewer side effects than synthetic ones. Therefore, further investigation is needed to identify the structural, sequence, and structure-activity relationship of polysaccharides from P. eryngii UN-1.

Acknowledgment

This study was supported by the Shandong Technology-based SMEs Innovation Ability Improvement Project (2021TSGC1281), the Horizontal Cooperation Project Foundation of Shandong University of Technology (9101-2223581). We are very grateful to Dr. Hassan Mohamed for polishing and modifying the language of the paper.

Author's Contributions

Qing Liu: Participated in project design and manuscript writing.

Haodong Wu, Ying Wu and Mengyu Liu: Participated in broth culture, extraction, and purification.

Yao Zhang: Participated in experiments on antioxidant and antimicrobial activity.

Yuanda Song: Participated in experimental guidance and manuscript revisement.

Ethics

All authors read and approved the final version of this manuscript. There are not any ethical issues to declare that could arise after the publication of this manuscript.

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