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ORIGINAL ARTICLE

Isolation, Structural Characteristics, and *in vitro* and *in vivo* Antioxidant Activity of the Acid Polysaccharide Isolated from *Pholiota nameko*

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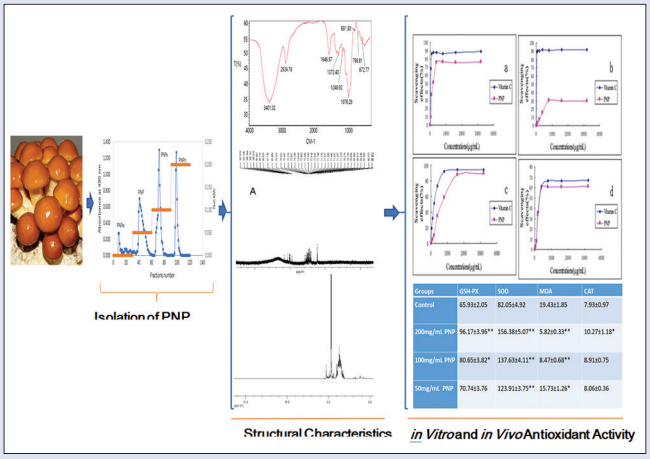
ABSTRACT

Background: Previous studies have revealed that edible fungi are rich in polysaccharides, which typically have strong biological activity. *Pholiota nameko* is one of the main cultivated edible fungi and an important economic crop. **Objectives:** In order to screen a polysaccharide with antioxidant activity from *Pholiota nameko*. **Methods and Materials:** A water-soluble acidic *P. nameko* polysaccharide (PNP) was found from extraction with alkaline water. After purification by chromatography, the structural features of PNP were analyzed by chromatographic, spectral, & nuclear magnetic resonance (NMR) spectroscopic analyses and the content of sulfate & uronic acid in PNP was determined concurrently. **Results:** The content of sulfate and uronic acid was 1.756 and 7.173 w/w, respectively. The molecular weight of PNP was 1.05 × 10^6 Da by gel permeation chromatography. The Fourier transform infrared (FTIR) and NMR spectra displayed that the following sugar chains might be present in PNP: β-1,4-D-Manp, →4)-β-D-Manp-(1→, β-1,3-D-Glcp, β-D-Xylp-(1→. Evaluation of in vitro antioxidant activity showed that PNP had a strong scavenging ability to scavenge ABTS+, hydroxyl, and superoxide anion radicals. The results of our in vivo experiments exposed that PNP might significantly lessen the formation of malondialdehyde. High levels of PNP can meaningfully increase the activity of catalase and superoxide dismutase activity in mice serum. In addition, PNP can significantly increase the activity of GSH-Px, effectively protect the structure and function of the cell membrane. **Conclusion:** PNP has antioxidant effect and can be explored as a potential antioxidant.

Key words: Acid polysaccharide, antioxidant, free radical, *Pholiota nameko*, structural characteristics

SUMMARY

- *Pholiota nameko* is a popular pleasant edible fungus and is extensively cultivated in China. It is opulent in polysaccharides, which display biological activity. In order to extraction and characterization of an acid polysaccharide with antioxidant activity from *Pholiota nameko*, a water-soluble acidic P. nameko polysaccharide (PNP) was found from extraction with alkaline water. The results exhibited that the content of sulfate and uronic acid was 1.756 and 7.173 w/w, respectively. The molecular weight was 1.05 × 106 Da. The following sugar chains might be present in PNP: β-1,4-D-Manp, →4)-β-D-Manp-(1→, β-1,3-D-Glcp, β-D-Xylp-(1→. Antioxidant activity showed that PNP had a strong scavenging ability to scavenge ABTS+, hydroxyl, and superoxide anion radicals, and might significantly lessen the formation of malondialdehyde, increase the activity of catalase, superoxide dismutase activity and GSH-Px.



Abbreviations Used: PNP: Polysaccharide of *Pholiota nameko*; IR: Infrared spectra; NMR: Nuclear Magnetic Resonance; OH•: Hydroxyl radicals; ABTS+: 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); CAT: Catalase; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; LPS: Lipopolysaccharide; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; HPGPC: High-performance Gel Permeation Chromatography; GC-MS: Gas chromatography–mass spectrometry.

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INTRODUCTION

Polysaccharides can produce more than 10 monosaccharide units after hydrolysis. It is extensively scattered across almost all organisms such as animals, plants, micro-organisms, and algae. It is one of the four basic substances establishing a life and is closely related to the upkeep of life function. It is used as an energy source and is a basic material of various organisms. However, the authors have established that polysaccharides from different sources have complex biological structures and that glycoconjugates are strictly related to many diseases, such as cancer

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and bacterial and viral infections.[1] Therefore, polysaccharides have attracted greater attention among various groups of researchers. Current studies have revealed that many polysaccharides are allied with various properties such as immunomodulatory effect,[2,3] antitumor effect,[4,5] antioxidant activity,[6] and anti-inflammatory activity[7] as a treatment for lupus nephritis,[8] and hypoglycemic activity,[9,10] and some active polysaccharides also play an vital role in preventing oxidative damage.[11,12]

Previous studies have exposed that when the level of free radicals increases, it can cause biofilm damage, protein denaturation, enzyme inactivation, and DNA replication errors, thereby accelerating cell aging and death.[13] Polysaccharides from edible mushrooms can scavenge free radicals, surge antioxidant enzyme activity, inhibit lipid peroxidation, protect biofilm from external stress, and deferral aging. Tian[14] found that water-soluble polysaccharides from *Houttuynia cordata* Thunb established good antioxidant activity.

Pholiota nameko is a popular pleasant edible fungus and is extensively cultivated in China. It is opulent in polysaccharides, which display hypolipidemic,[15] anti-inflammatory,[16] and immunological effects.[17] It also comprises proteins that show antitumor activity.[18] However, we intended to extract, isolate, and conduct a structural analysis of the acidic polysaccharide from *P. nameko* (PNP) and assess its antioxidant capacity under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Materials and chemicals

Dextrans were purchased from Pharmacia Co. (Uppsala, Sweden). AB-8 and ADS-7 were procured from the Chemical Plant of Nankai University (Tianjin, China). 1,1-Diphenyl-2-picrylhydrazine (DPPH) radical; lipopolysaccharide (LPS); 2,2-azobis (2-methylpropionamidine)-dihydrochloride (AAPH); trolox; 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide (MTT); and Vitamin C were procured from Sigma (St. Louis, USA). 2,2'-azinobis(3-ethylbenzthiazoline) 6-sulphonic acid (ABTS) was procured from Merck, (Darmstadt, Germany). Ethanol, petroleum ether, pyridine, hydroxylamine hydrochloride and all other chemicals and reagents were of analytical grade.

Extraction and purification of *Pholiota nameko* polysaccharide

PNP was extracted according to the method of Xiao *et al.*[19] First, 50 g of the powder of *P. nameko* was added into a round-bottomed flask with 500 mL petroleum ether (b.p. 60°–90°), and the mixture was refluxed in a water bath for 2 h, filtered, and the filtrate was dried until a continuous mass was found. To the dried residue, 0.2 M sodium hydroxide (500 mL) was added to extract the polysaccharide and mixed at room temperature for 6 h. Then, the filtrate was collected and neutralized by adding 0.2 M hydrochloric acid (500 mL). The protein was extracted by Sevage reagent (*n*-butanol: trichloromethane = 1:4) for four times.[20] The solvent is concentrated to 100 mL, and anhydrous ethanol is added slowly until the volume of ethanol in the system reaches 70%. Finally, the precipitate was collected by centrifugation.

The crude PNP was more purified based on the method described by Luo *et al.*[21] with minor alterations. First, the polysaccharide was dissolved in deionized water, filtered with 0.45 μm nucleolar pore, and purified on AB-8 macroporous resin column (26 mm × 300 mm) to eliminate the pigment. Next, the eluant was purified with ADS-7 ion exchange resin (26 mm × 300 mm) to confiscate the residual protein. Then, 80 mg of crude PNP was dissolved in 8 mL of ddH_2O and loaded onto DEAE-cellulose column and then serially eluted with ddH_2O, 0.05 M NaCl, 0.1 M NaCl, and 0.2 M NaCl solution and the eluate was collected by the fully automatic partial collector. A total of 30 tubes (6 mL each, elution speed 1 mL/min) were collected for each elution gradient. The polysaccharide content was observed by the sulfuric acid phenol method. The purified PNP samples were collected based on the elution peak (PNPw, PNP, PNPx, and PNPn were obtained by gradient elution). Finally, the purified sample was dialyzed with deionized water for 72 h, frozen, and dried.

Structural characteristics of *Pholiota nameko* polysaccharide

The content of protein in PNP was evaluated according to the method of Bradford.[22] The total flavone content in PNP was determined by $AlCl_3$ method described by Zuo *et al.*[23] The total phenolic content was analyzed by the Folin phenol method based on the explanation.[24] The content of sulfate radical in the PNP was assessed by the phenol sulfuric acid method with K_2SO_4 as the standard.[25] The uronic acid content was analyzed by carbazole sulfuric acid method using D-glucuronic acid as the standard.[26]

The molecular weight (MW) of PNP was determined by using the high-performance gel permeation chromatography (HP-GPC) according to the Yamamoto method.[27] Samples and series of standard dextran solutions (2500; 4600; 7100; 10,000; 21,400; 41,100; 84,400; 133,800; 200,000; 555,500; 1,110,000; and 2,990,000 Da) were analyzed by HP-GPC using refractive detector. The analytical columns were Ultrahydrogel™ 2000 and Ultrahydrogel™ 250 (ID: 300 × 7.8 mm) in series at room temperature. We used 0.2 mol/L phosphate buffer solution as the mobile phase with a flow rate of 0.7 mL/min.

The structural features of PNP were measured by Fourier-transform infrared spectroscopy (FTIR).[28] Briefly, 1.0 mg PNP was ground consistently with KBr powder at a ratio of 1:20 (W: W) and then scanned by using FTIR spectrophotometer (Perkin-Elmer Corp., USA) in the scanning range of 4000–500 cm^{-1}. The monosaccharide composition in the PNP was analyzed by GC-MS, ^{1}H-NMR, and ^{13}C-NMR spectral analysis according to the method defined by Luo.[29]

In vitro antioxidant activity

ABTS free-radical scavenging assay

In this experiment, the free-radical scavenging activity of PNP against $ABTS^+$ was determined according to the method of Re[30] with slight alterations. ABTS powder (0.0192 g) was weighed in a centrifuge tube and 5 mL of phosphate-buffered saline (PBS) (0.01 mol/L) was added to dissolve it. After this, potassium persulfate (0.0033 g) was precisely weighed and added to it and entirely dissolved. The tube was closed and placed in dark for 16 h at room temperature for $ABTS^+$ to be formed. 100 mL of the reaction mixture was withdrawn and diluted by adding 0.01 mol/L PBS to the solution so that the absorbance value at 734 nm was 0.70 + 0.02. The diluent reacts at room temperature for 30 minutes. Standard solution of Vitamin C (0.1 mL) and PNP were added into the test tube; blank control was prepared with the same amount of deionized water. Next, 1.0 mL of ABTS solution (A_{734} = 0.70 + 0.02) was added to each test tube and permissible to react at room temperature for 20 min. The absorbance of the solution at 734 nm was assessed by ultraviolet spectrophotometer. The scavenging effects were considered based on the following formula:

ABTS radical scavenging activity (%) = [Ac − (A − Ab)]/Ac × 100.

Where Ac is the absorbance of ABTS solution without the added sample at 734 nm, Ab is the absorbance of sample without the addition of ABTS, and A is the absorbance of sample and ABTS.

DPPH free-radical scavenging activity

DPPH free-radical scavenging capability of various fractions was assessed using the method of Fan *et al.*[31] with some alterations. Briefly, 0.3 mL of standard solution (Vitamin C) and PNP solution with various concentrations (50, 100, 200, 400, 800, 1600, and 3200 μg/mL) were taken in the test tube; blank control was prepared by adding the same amount of deionized water. Then, 0.9 mL of DPPH methanol solution (0.1 mmol/L) was added to each test tube and the reaction was conducted at room temperature for 30 min. After the reaction, the absorbance of the reaction mixture was read at 517 nm by UV-Vis spectrophotometer. The DPPH free-radical scavenging ability was considered as follows:

DPPH radical scavenging activity (%) = [Ac − (A − Ab)]/Ac × 100.

Where Ac is the absorbance of DPPH without sample at 517 nm, Ab is the absorbance of the sample without DPPH, and A is the absorbance of sample and DPPH.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of PNP samples was measured according to the method of Luo *et al.*[32] Briefly, 0.3 mL PNP and Vitamin C at with various concentrations (50, 100, 200, 400, 800, 1600, and 3200 μg/mL) were incubated with 0.5 mL of EDTA–Fe (2 mM), 1.0 mL of H_2O_2 (3% v/v), and 0.36 mg/mL crocus in 4.5 mL sodium phosphate buffer (150 mM, pH 7.4) for 35 min at 37°C and the hydroxyl radicals were distinguished by monitoring the absorbance at 520 nm. The hydroxyl radical scavenging activity of samples was considered as follows:

Hydroxyl radical scavenging activity (%) = [(Ac − A)/Ac] × 100.

Where Ac is the absorbance of the control solution and A is the absorbance of the sample solution.

Superoxide anion free-radical scavenging activity

Determination of superoxide anion radical scavenging activity of PNP was conducted according to the method described by Wang *et al.*[33] with minor alterations. Briefly, 4.5 mL of 50 mmol/L Tris-HCl (pH 8.2) and 1.0 mL of PNP or Vitamin C at various concentrations (50, 100, 200, 400, 800, 1600, and 3200 μg/mL) were mixed in tubes. The reaction mixture was incubated for 20 min at 25°C. At the same time, 0.4 mL of pyrogallol (25 mmol/L) was added. After 4 min, the reaction was completed by adding 0.1 mL of 8.0 mol/L HCl solution. The absorbance of the sample was assessed by the UV-Vis spectrophotometer at 325 nm. The scavenging activity of PNP against superoxide anion was examined by the following equation:

Superoxide anion scavenging activity (%) = (Ac − A)/Ac × 100.

Where Ac is the absorbance without sample and A is absorbance with a sample at 325 nm.

In vivo antioxidant activity

Specific-pathogen-free Kunming mice (male: female = 50:50, provided by Sichuan Academy of Medical Sciences) weighing 18–22 g were employed in this study. The mice were randomly separated into four groups: control group (normal saline group), high-concentration PNP group (200 mg/mL), medium-concentration PNP group (100 mg/mL), and low-concentration PNP group (50 mg/mL). After 28 days, the mice were forfeited and serum from the eye socket was used to observe the concentrations of GSH-PX, superoxide dismutase (SOD), and malondialdehyde (MDA).

Statistics analysis

The test results were articulated as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS software version 23.0 and significant difference, very significant difference, and extremely significant difference were uttered by $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

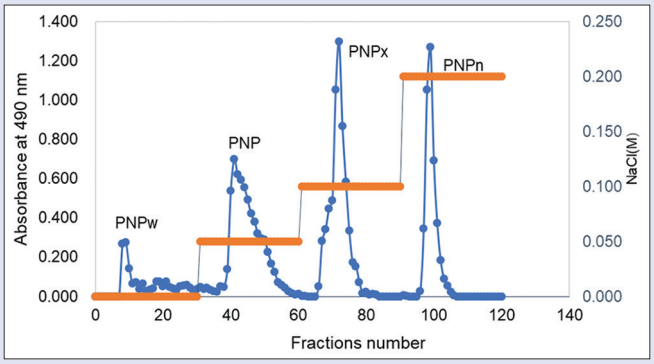

Figure 1: Isolated of crude polysaccharide by DEAE-cellulose column (26 mm × 300 mm)

RESULTS

Preparation and structural characteristics of *Pholiota nameko* polysaccharide

The polysaccharides PNPw, PNP, PNPx, and PNPn were gotten by gradient elution of ultrapure water, 0.05 M NaCl, 0.1 M NaCl, and 0.2 M NaCl on DEAE-cellulose column (26 mm × 300 mm) [Figure 1]. PNP was found as a gray powder after purification from the macroporous adsorption resin and ion-exchange chromatography; the crop was 5.8%. According to the results, PNP did not encompass phenols and flavonoids. However, it satisfied of sulfuric radical (1.756 w/w) and uronic acid (7.173 w/w) in PNP.

The MW of PNP is carefully related to its biological activity. If the MW is too big, it may damagingly disturb the solubility of PNP and its biological activity. Therefore, we verified the MW of PNP by comparing it with the standard curve of dextran attained by high-performance gel chromatography. Based on this, the MW of PNP was found to be 1.05×10^6 Da.

Infrared spectrum of PNP [Figure 2] demonstrations that the absorption peak of –OH group of the sugar molecule was recorded at 3401 cm^{-1}, the absorption peak of C-H was recorded at 2924 cm^{-1}, and the absorption peak of C = O was recorded at 1646 cm^{-1}, which displays that PNP is a polysaccharide in nature. The characteristic absorption at 891 cm^{-1} indicates the β-configuration of the sugar units.[34] In addition, there were two absorption peaks between 1120 and 1010 cm^{-1}, which shows a furanose form of sugar in PNP. From the infrared spectrum of PNP, it can be contingent that it is mainly unruffled of β-form of furanose.

We found four peaks for the standard monosaccharides in the GC-MS spectrum of PNP, among which the peak area of glucose derivative was the uppermost, representing that glucose is the most important monosaccharide in PNP. The peak area of mannose is the second largest, signifying that the content of mannose is great. However, the peak area of xylose was smaller, demonstrating that the content of PNP is fewer. According to the calculation of peak area, the molar ratio of four monosaccharades in PNP is as trails: mannose: glucose: galactose: xylose = 28.05 mol%:41.23 mol%:16.92 mol%:3.86 mol%.

This demonstrates the monosaccharide type of PNP, the configuration of glycosidic bond, and the position of sugar connection from carbon spectrum and hydrogen spectrum [Figure 3a and b]. In ^{1}H-NMR, the proton signal of heterocyclic carbon is mostly concentrated in the region less than δ5.0 ppm, designating that the sugar molecules in PNP were

mainly of β configuration. However, there were also some absorption peaks in the region greater than δ5.0 ppm, specifying that a small amount of sugar molecules in PNP was of α-configuration.[35,36] The signal of δ102.55 ppm in ^{1}H-NMR and δ4.53 in ^{13}C-NMR presented that the glucose residue in PNP was mostly of β-configuration. The absorption peaks at δ3.88 ppm and δ3.75 ppm in ^{1}H-NMR are typical signals of mannose. The absorption peaks at δ4.532 ppm and δ3.28 ppm in ^{1}H-NMR are typical signals of glucose. The absorption peaks at δ3.546 ppm and δ3.88 ppm in ^{1}H-NMR are typical signals of galactose. The absorption peak at δ4.7 ppm in ^{1}H-NMR is β-D-Xylp-1 → heterocephalic proton.[37] There is no absorption peak in the ^{13}C-NMR spectrum from δ61 ppm to δ68 ppm, demonstrating that there is no 1 → 6 bond in PNP. ^{13}C-NMR spectra show absorption peaks in the range of δ70.7 ppm to δ73 ppm, indicating that there is a C4 bond in PNP. In ^{13}C-NMR spectra, there are absorption peaks in the range of δ76.8 ppm to δ80 ppm, showing that PNP has branching at C3 and C4 positions and there may be 1 → 3 and 1 → 4 bonds.[38] Based on the articles, we can say that the above-mentioned chains are the main sugar chains in PNP [Table 1].

In vitro antioxidant activities

The free-radical scavenging activity of PNP was verified on superoxide anion, hydroxyl, DPPH, and ABTS radicals [Figure 4]. The scavenging capacities of PNP were found to be related to its concentration, viewing a certain concentration dependence.

In the ABTS free-radical experiment [Figure 4a], the positive control (Vitamin C) presented a strong dose-dependent scavenging activity. The maximum scavenging was recorded at 3200 μg/mL (89.9%). PNP also established a good scavenging effect on ABTS free radicals. The effect was not clear at lower concentration but augmented with increasing concentration. The maximum outcome was recorded at 3200 μg/mL (76.5%), which was close to that of the positive control. Thus, we can say that PNP displays a significant effect on ABTS radical scavenging ($P < 0.05$).

Figure 4b displays the scavenging effect of PNP on DPPH free radicals. The positive control (Vitamin C) and PNP exhibited dose-dependent scavenging ability. With the increase in the concentration, the scavenging activity of each sample presented an upward trend. The maximum activity was recorded at 100 μg/mL for Vitamin C (90.5%). On the contrary, the scavenging ability of PNP on DPPH radical was not noteworthy ($P > 0.05$). Even at a high concentration (3200 μg/mL), its scavenging capability was only 30.1%. Thus, we can say that PNP has no significant scavenging effect on DPPH free radicals.

Figure 4c confirms the scavenging activity of Vitamin C against hydroxyl radicals. We established low concentrations (0–800 μg/mL) and high concentrations (800–3200 μg/mL) and the maximum activity was recorded at 3200 μg/mL (94.5%). PNP established slowly increasing activity in the range of 0–1600 μg/mL and reached the maximum value (98.6%) at 3200 μg/mL ($P < 0.05$). There was no important difference between PNP and Vitamin C. According to our results, PNP proved significant scavenging ability against hydroxyl radicals.

Figure 4d expresses the results of scavenging superoxide anions [Figure 4d]. According to our results, the scavenging effect of PNP

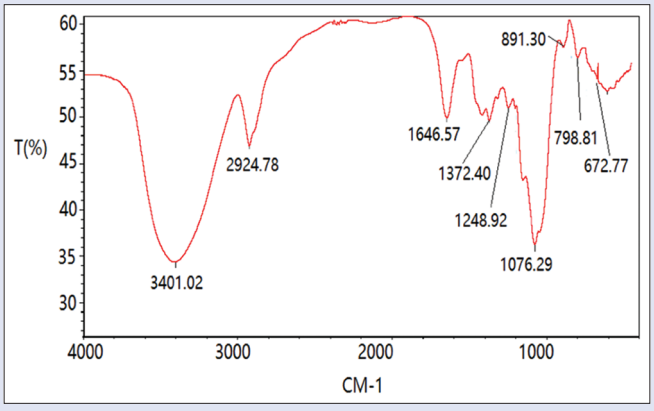

Figure 2: Fourier-transform infrared spectra of the polysaccharide *Pholiota nameko* polysaccharide

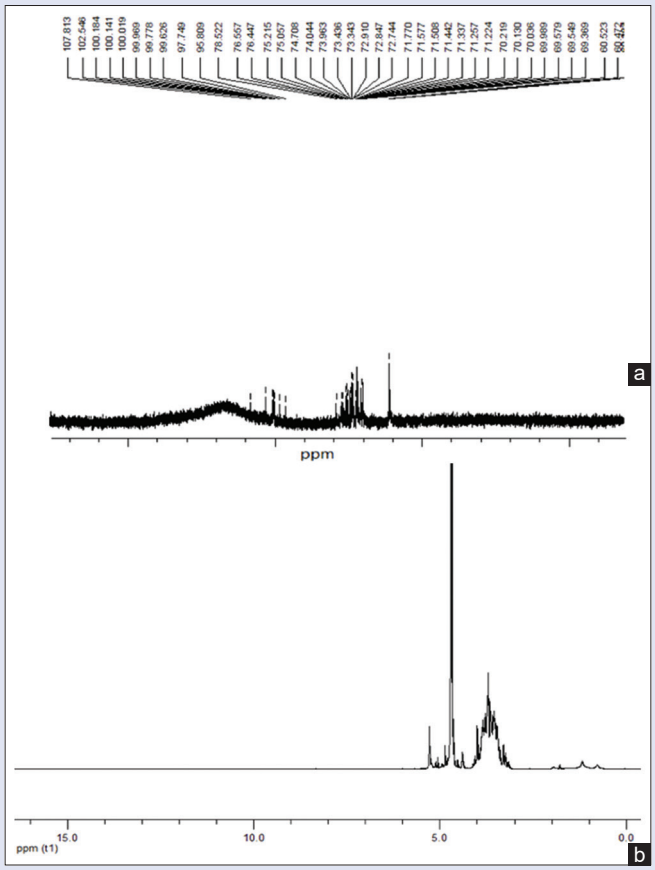

Figure 3: Nuclear magnetic resonance test of *Pholiota nameko* polysaccharide. (a) For ^{13}C-NMR test of *Pholiota nameko* polysaccharide and (b) for ^{1}H-nuclear magnetic resonance test of *Pholiota nameko* polysaccharide

Table 1: The chemical shifts (^{1}H nuclear magnetic resonance and ^{13}C nuclear magnetic resonance) of the polysaccharide *Pholiota nameko* polysaccharide

Sugar residues	H-1; C-1	H-2; C-2	H-3; C-3	H-4; C-4	H-5; C-5	H-6; C-6
β-1,4-D-Manp	4.70; 102.55	3.88; 70.22	3.82; 75.22	3.65; 78.52	3.70; 76.45	3.98, 3.66; 60.47
→4)-β-D-Manp-(1→	4.532; 97.73	4.00; 71.22	3.75; 71.77	3.78; 76.56	3.70; 76.45	3.94; 60.52
β-1,3-D-Glcp	4.70; 102.55	3.24; 72.91	3.73; -	3.28; 69.55	3.546; 74.71	3.88, 3.66; 60.52
β-D-Xylp-(1→	4.70; 102.55	3.28; 73.96	3.55; 76.45	3.65; 70.04	3.24; -	

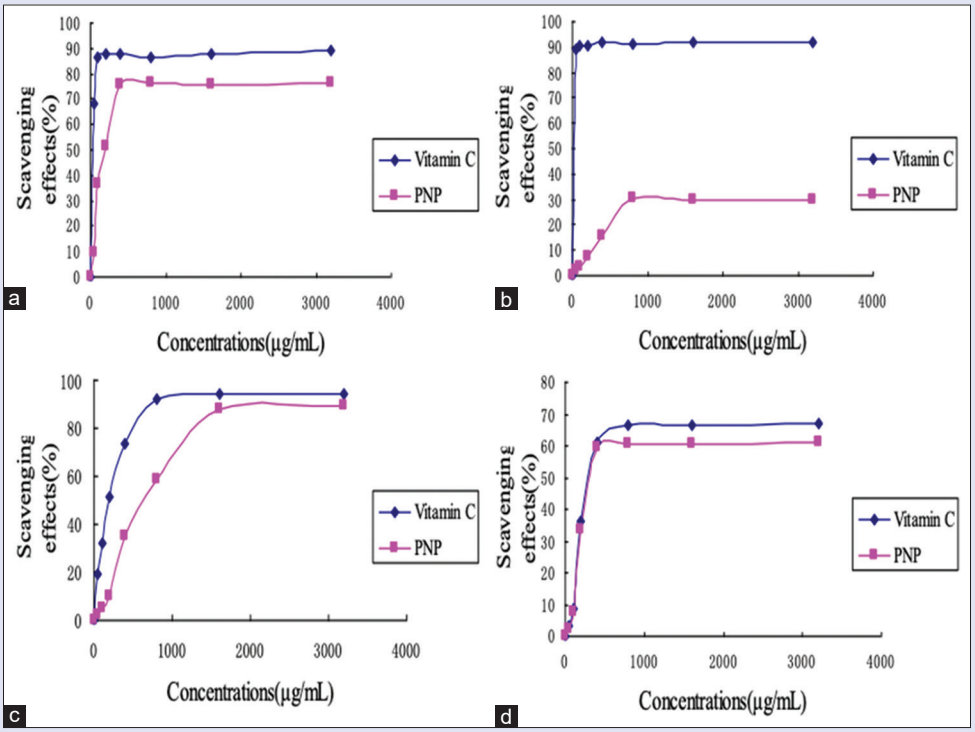

Figure 4: Antioxidant activities of *Pholiota nameko* polysaccharide *in vitro*. (a) The scavenging effects of *Pholiota nameko* polysaccharide on ABTS radicals, (b) The scavenging effects of *Pholiota nameko* polysaccharide on DPPH radicals, (c) The scavenging effects of *Pholiota nameko* polysaccharide on OH radicals, (d) The scavenging effects of *Pholiota nameko* polysaccharide on superoxide anion radicals

Table 2: Antioxidant activities of the *Pholiota nameko* polysaccharide *in vivo*

Groups	GSH-PX	SOD	MDA	CAT
Control	65.93±2.05	82.05±4.92	19.43±1.85	7.93±0.97
200 mg/mL PNP	96.17±3.96**	156.38±5.07**	5.82±0.33**	10.27±1.18*
100 mg/mL PNP	80.65±3.82*	137.63±4.11**	8.47±0.68**	8.91±0.75
50 mg/mL PNP	70.74±3.76	123.91±3.75**	15.73±1.26*	8.06±0.36

**$P < 0.01$, *$P < 0.05$ vs. control. MDA: Malondialdehyde; CAT: Catalase; PNP: Polysaccharide from *Pholiota nameko*; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase

and Vitamin C is comparable. With the increase in the concentration, the scavenging activity gradually upsurges. There was no statistically significant difference between the scavenging effect of PNP and Vitamin C at 400 μg/mL concentration ($P > 0.05$), indicating that PNP had a very sturdy superoxide anion scavenging activity.

Antioxidant activities *in vivo*

GSH-Px is a vital antioxidant enzyme, which scavenges hydrogen peroxide *in vivo*. It can also precisely catalyze the reduction of reduced glutathione to hydrogen peroxide and defend the structure and function of the cell membrane. As shown in Table 2, compared to the control, 100 mg/mL PNP suggestively augmented the content of GSH-PX in the serum of mice ($P < 0.05$). However, at low concentrations (50 mg/mL), there was no significant change between PNP and the control. With the increase in the concentration, the effect of PNP on GSH-PX increased progressively. The maximum activity was recorded at 200 mg/mL. PNP might suggestively recover the activity of GSH-PX ($P < 0.01$).

SOD mostly scavenges superoxide anion radicals under *in vivo* conditions. The reduction in the activity of SOD might lead to lipid peroxidation and deposition of lipofuscin in cells, resultant in cellular dysfunction. Table 2 demonstrates that PNP might significantly increase the activity of SOD in mice ($P < 0.05$). Compared with the control group, PNP expressively increased the activity of SOD at low concentration (50 mg/mL) ($P < 0.05$). With the increase in PNP levels, the effect of PNP on the activity of SOD is superior.

Hydrogen peroxide can induce lipid peroxidation of cell membranes, abolish the structure of cell membranes, induce apoptosis, and contribute in a variety of pathological mechanisms. Catalase (CAT) can exactly eliminate hydrogen peroxide from the body and avoid oxidative damage. According to our results, a high concentration of PNP might significantly increase the activity of CAT ($P < 0.05$), but a medium-to-low concentration of PNP had no significant consequence on CAT activity.

MDA is an imperative metabolite in the process of lipid peroxidation. It is produced by the oxidation of unsaturated fatty acids on the cell membrane under the direct action of radiation and hydroxyl radical. Therefore, the quantity of MDA formed can specify the degree of lipid peroxidation and it is one of the vital indicators to judge the damage of free radicals. The results showed that compared with the control group, low concentration of PNP may intentionally remove MDA in mouse serum, and the higher the concentration of PNP, the more obvious the effect of removing MDA.

CONCLUSION

In this study, PNP was prepared by alkali water extraction and ethanol precipitation. The content of the sulfuric radical and uronic acid in the polysaccharide PNP was confirmed, after purifying it on a macroporous resin. According to the findings, the level of sulfuric radical was 1.756 w/w% and the level of uronic acid was 7.173 w/w%. The MW of PNP was 1.05×10^6 Da using the HPGPC method. The results of GC-MS display that PNP comprises four monosaccharides and glucose is the most plentiful monosaccharide in PNP. The molar ratio of four

monosaccharides in PNP was as follows: mannose: glucose: galactose: xylose = 28.05 mol%:41.23 mol%:16.92 mol%:3.86 mol%. The outcomes of Fourier-transform infrared spectroscopy indicate that PNP chiefly checked furanose in the β-configuration form and that there may be the following sugar chains in PNP: s β-D-Xylp-(1 → heterocephalic proton,β-1,4-D-Manp, →4)-β-D-Manp-(1→,β-1,3-D-Glcp, β-D-Xylp-(1→.

PNP displayed a resilient scavenging activity against ABTS free radicals, hydroxyl free radicals, and superoxide anion free radicals under *in vitro* circumstances. The antioxidant effect of PNP was comparable to that of Vitamin C. However, the DPPH scavenging activity of PNP was fragile.

In vivo findings established that PNP significantly abridged the content of MDA in mice compared with the control group, signifying that PNP scavenges lipid peroxidation products under *in vivo* conditions. High concentrations of PNP can significantly increase the activity of CAT and SOD in mice, which augments the role of endogenous free-radical scavenging system and lessens the production of lipid peroxides. At the same time, PNP can suggestively increase the activity of GSH-PX and effectively protect the structure and function of cell membrane, which displays that PNP has antioxidant effect *in vivo*.

Previous studies have revealed that many plant polysaccharides have immunopotentiating activity and *P. nameko* polysaccharides can significantly progress the immune function of tumor mice.[39] Concurrently, there are reports on polysaccharides with regard to their antioxidant activity and protection of cell membrane integrity. Some polysaccharides can struggle with the damage of immune cells induced by reactive oxygen species and play a part in the immunoregulation by refining the antioxidant activity of enzymes in immune cells and preserving the integrity of their cell membrane. In this study, PNP confirmed antioxidant activity under *in vitro* and *in vivo* conditions and played an important part in increasing the immunomodulatory ability. Thus, it can be employed as a potential antioxidant molecule.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ueno M, Nogawa M, Siddiqui R, Watashi K, Wakita T, Kato N, *et al.* Acidic polysaccharides isolated from marine algae inhibit the early step of viral infection. Int J Biol Macromol 2019;124:282-90.
2. Pu YW, Liu ZJ, Tian H, Bao YY. The immunomodulatory effect of poria cocos polysaccharides is mediated by the Ca2+/PKC/p38/NF-κB signaling pathway in macrophages. Int Immunopharmacol 2019;72:252-57.
3. Ma L, Jiao K, Luo L, Xiang J, Fan J, Zhang X, *et al.* Characterization and macrophage immunomodulatory activity of two polysaccharides from the flowers of *Paeonia suffruticosa* andr. Int J Biol Macromol 2019;124:955-62.
4. Faten H, Amira E, Elshahat T, Mohammed A. Antitumor effect of a crude protein-bound polysaccharide substance extracted from volvariella speciose. Bioactive Carbohydrates and Dietary Fibre 2018;16:75-81.
5. Chen YY, Xue YT. Optimization of microwave assisted extraction, chemical characterization and antitumor activities of polysaccharides from porphyra haitanensis. Carbohydr Polym 2019;206:179-86.
6. Zhao Y, Hu W, Zhang H, Ding C, Huang Y, Liao J, *et al.* Antioxidant and immunomodulatory activities of polysaccharides from the rhizome of dryopteris crassirhizoma nakai. Int J Biol Macromol 2019;130:238-44.
7. Zhang Y, Pan X, Ran S, Wang K. Purification, structural elucidation and anti-inflammatory activity *in vitro* of polysaccharides from smilax china L. Int J Biol Macromol 2019;139:233-43.
8. Jiang X, Zhao X, Luo H, Zhu K. Therapeutic effect of polysaccharide of large yellow croaker swim bladder on lupus nephritis of mice. Nutrients 2014;6:1223-35.
9. Wu GJ, Liu D, Wan YJ, Huang XJ, Nie SP. Comparison of hypoglycemic effects of polysaccharides from four legume species. Food Hydrocolloid 2019;9:299-304.
10. Jong WY, Sang WK, Hye JH. Hypoglycemic effect of fungal polysaccharides in ob/ob mice and molecular mechanism study by proteomic analysis. J Biotechnol 2007;131:55.
11. Wu Q, Zheng C, Ning ZX, Yang B. Modification of low molecular weight polysaccharides from tremella fuciformis and their antioxidant activity *In vitro*. Int J Mol Sci 2007;8:670-79.
12. Tsiapali E, Whaley S, Kalbfleisch J, Ensley HE, Browder IW, Williams DL, *et al.* Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. Free Radic Biol Med 2001;30:393-402.
13. Liu X, Hou R. Wang D. Comprehensive utilization of edible mushroom Auricularia auricula waste residue-extraction, physicochemical properties of melanin and its antioxidant activity. Food Sci Nutr 2019;7:3774-83.
14. Tian LM, Zhao Y, Guo C. A comparative study on the antioxidant activities of an acidic polysaccharide and various solvent extracts derived from herbal *Houttuynia cordata*. Carbohydr Polym 2011;83:537-44.
15. Li H, Zhang M, Ma G. Hypolipidemic effect of the polysaccharide from *Pholiota nameko*. Nutrition 2010;26:556-62.
16. Li H, Lu X, Zhang S, Lu M, Liu H. Anti-inflammatory activity of polysaccharide from *Pholiota nameko*. Biochemistry (Mosc) 2008;73:669-75.
17. Li HP, Tao YQ, Zhao P, Huai LH, Zhi DX, Liu JiM, *et al.* Effects of *Pholiota nameko* polysaccharide on NF-κB pathway of murine bone marrow-derived dendritic cells. Int J Biol Macromol 2015;77:120-30.
18. Zhang Y, Liu Z, Ng TB, Chen Z, Qiao W, Liu F, *et al.* Purification and characterization of a novel antitumor protein with antioxidant and deoxyribonuclease activity from edible mushroom *Pholiota nameko*. Biochimie 2014;99:28-37.
19. Xiao HW, Cai XR, Fan YJ, Luo AX. Antioxidant activity of water-soluble polysaccharides from brasenia schreberi. Pharmacogn Mag 2016;12:193-97.
20. Navarini L, Gilli R, Gombac V, Abatangelo A, Bosco M, Toffanin R. Polysaccharides from hot water extracts of roasted coffea arabica beans: Isolation and characterization. Carbohydr. Polym 1999;40:71-81.
21. Luo AX, He XJ, Zhou SD, Fan YJ, Luo AX, Chun Z. Purification, composition analysis and antioxidant activity of the polysaccharides from dendrobium nobile Lindl. Carbohydr. Polym 2010;79:1014-19.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
23. Zuo LL, Wang ZY, Fan ZL, Tian SQ, Liu JR. Evaluation of antioxidant and antiproliferative properties of three actinidia (*Actinidia kolomikta*, *Actinidia arguta*, *Actinidia chinensis*) extracts *in vitro*. Int J Mol Sci 2012;13:5506-18.
24. Sim KS, Nurestri AM, Norhanom AW. Phenolic content and antioxidant activity of crude and fractionated extracts of pereskia bleo (Kunth) DC.(Cactaceae). Afri J Pharm Pharmacol 2010;4:193-201.
25. Dodgson KS, Price RG. A note on the determination of the ester sulphate concent of sulphated polysaccharides. Biochem J 1962;84:106-10.
26. Lin Y, Huang LJ, Tian GY. An improved method for the quantitative determination of uronic acid. Chin Herbal Med 1999;30:817-19.
27. Yamamoto Y, Nunome T, Yamauchi R, Kato K, Sone Y. Structure of an exocellular polysaccharide of lactobacillus helveticus TN-4, a spontaneous mutant strain of lactobacillus helveticus TY1-2. Carbohydr Res 1995;275:319-32.
28. Kiho T, Sobue S, Ukai S. Structural features and hypoglycemic activities of two polysaccharides from a hot-water extract of agrocybe cylindracea. Carbohydr Res 1994;251:81-7.
29. Luo A, He X, Zhou S, Fan Y, He T, Chun Z, *et al. In vitro* antioxidant activities of a water-soluble polysaccharide derived from dendrobium nobile lindl. Extracts. Int J Biol Macromol 2009;45:359-63.
30. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231-7.
31. Fan YJ, Ge ZF, Luo AX. *In vitro* antioxidant activity of polysaccharide from gardenia jasminoides Ellis.J Med Plants Res 2011;5:2963-68.
32. Luo AX, Fan YJ, Luo AS. *In vitro* free radicals scavenging activities of polysaccharide from

Polygonum Multiflorum Thunb. J Med Plants Res 2011;5:966-72.

33. Wang J, Zhang Q, Zhang Z, Li Z. Antioxidant activity of sulfated polysaccharide fractions extracted from laminaria japonica. Int J Biol Macromol 2008;42:127-32.
34. Coimbra MA, Goncalves F, Braaos AS, Delgadillo I. Fourier transform infrared spectroscopy and chemometric analysis of white wine polysaccharide extracts. J Agr Food Chem 2002;50:3405-11.
35. Roy SK, Maiti D, Mondal S, Das D, Islam SS. Structural analysis of a polysaccharide isolated from the aqueous extract of an edible mushroom, pleurotus sajor-caju, cultivar Black Japan. Carbohyd Res 2008;343:1108-13.
36. Yoon S, Kim MK, Lee IY, Yun M, Nam Shin JE. Production and structural features of a water-soluble polysaccharide from a mutant strain of Agrobacterium sp. J Ind Eng Chem 2008;14:759-64.
37. Duus J, Gotfredsen CH, Bock K. Carbohydrate structural determination by NMR spectroscopy: Modern methods and limitations. Chem Rev 2000;100:4589-614.
38. Xu CP, Sun YY, Bai JF, Jia XW, Ma KY, Wang XJ, *et al.* Study on the extraction, sulfation modification and antionxidant activity of yam polysaccharides. J Henan University of Technol (In chinese) 2019;40:50-55.
39. Chen J, Xiang Y. Immunological and antitumor activities of the polysaccharides from *Pholiota nameko*. Modern Food Sci Technol 2013;29:1800-04.