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Full Length Article

Structural Elucidation and Antioxidant Activities of Polysaccharides from *Pleurotus ferulae*

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Abstract

A polysaccharide was isolated and purified from fruiting body of *Pleurotus ferulae* Lenzi and named PFLP (*Pleurotus ferulae* Lenzi polysaccharide). An investigation of its structural characteristics and antioxidant activities showed that PFLP was composed mainly of rhamnose, mannose, xylose, galactose and glucose with an average molecular weight of 10,100 Da. PFLP was a triple helical polysaccharide composed mainly of β-(1→6)-glucosyl and β-(1→3)-D-glucan. The antioxidant effect of PFLP was shown in its high hydroxyl radical, ABTS and DPPH scavenging activity, as well as its moderate superoxide radical scavenging activity. However, compared to other fungal polysaccharides, PELP showed lower chelating activity for Fe^{2+} and reducing power. The results indicated that PFLP may be useful as an antioxidant instead of those supplied by chemical synthesis. © 2019 Friends Science Publishers

Keywords: Antioxidant activity; Chemical characteristics; *Pleurotus ferulae*; Polysaccharide

Introduction

Polysaccharides exist widely in nature. They can be found in plants, animals, mushrooms and algae. In recent years, numerous studies have shown that polysaccharides are substances that store energy for organisms to participate in life activities (Liu *et al.*, 2014; Chen *et al.*, 2018; Hu *et al.*, 2018). In addition, many studies have shown that polysaccharides have prominent biological activities, such as immunomodulatory (Saadat *et al.*, 2019), antioxidant (Qin *et al.*, 2018), anticancer (Chen *et al., 2018*), antitumor (Chen *et al.*, 2018) and anti-diabetic activity (Hu *et al.*, 2018). For example, the antioxidant activity includes scavenging of DPPH radicals, hydroxyl radicals, superoxide anions and the reducing power of *Cereus cladodes* polysaccharides was determined by spectrophotometry, showing that these polysaccharides had remarkable antioxidant activity (Petera *et al.*, 2015). Some bacterial (*Sphingomonas elodea* ATCC 31461) exopolysaccharides have been assayed for their ability to scavenge DPPH radicals *in vitro* (Qin *et al.*, 2018). As a result, polysaccharides have been gained increasing recognition in biotechnology (Delattre *et al.*, 2015). Notably, polysaccharides from edible mushrooms have interested scientists due to their various beneficial properties (Qin *et al.*, 2018). Edible mushroom polysaccharides have been widely used. In the food industry, edible mushroom polysaccharides are often used as grinding agents, thickeners, stabilizers, suspending agents, emulsifiers and flavor enhancers. In the pharmaceutical industry, polysaccharides are often made into drugs to improve immune function and alleviate symptoms of liver and kidney diseases (Dang *et al.*, 2013). Presently, many polysaccharide fractions have been isolated, purified, and their structures identified for different applications (Hu *et al.*, 2018; Qin *et al.*, 2018; Saadat *et al.*, 2019). Many polysaccharides isolated from different mushrooms have different structures and biological activities. As reported, polysaccharides from edible mushrooms consist of a backbone of β-linked glucans mostly and the structure and chain conformation influences its biological activity (Dang *et al.*, 2013). Because of the wide range of applications of polysaccharides from edible mushrooms, the importance of studying the structure and the biological activities cannot be overstated.

Pleurotus ferulae Lenzi is a mushroom that belongs to the *Basidiomycota, Hymenomycetes, Agaricales, Pleurotaceae, Pleurotus*. Growing in arid grasslands in Xinjiang China, it is considered to be a traditional edible and medicinal fungus because of its high nutritional value and delicious taste on. The chemical constituents of *Pleurotus* fungi mainly belong to polysaccharides that have

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good biological activity and health characteristics. Extensive studies have been conducted on polysaccharides from different sources; however, few have been studied *P. ferulae*. The objective of this work was to investigate the characterization and antioxidant activity of PFLP, aiming to clarify the relationship between the structure and antioxidant activity of PFLP.

Materials and Methods

Experimental Details and Treatments

P. ferulae was acquired from Qinghe County (Xinjiang, China). DEAE-52 cellulose and Sephadex G-100 were quired from Sigma-Aldrich (U.S.A.). Dextran standards were acquired from Showa Denko (Japan). Chemicals and reagents used in this study were all of analytical grade.

Preparation of Polysaccharide

Fresh fruiting bodies of *P. ferulae* (500 g) were homogenized at 80ºC and ground to a fine powder. 100 g of the powder was placed in distilled water for 40 min at 65ºC at a 1:25 ratio of powder to water. The supernatant was collected after centrifugation at 5000 rpm for 10 min and concentrated to one-fourth of the original volume. This was then mixed with 5 volumes of ethanol and stored at 4ºC for 12 h. The precipitate was obtained after centrifugation at 5000 rpm for 10 min and was completely solubilized in ultrapure water, washed four times with ethanol, and the mixture was then deproteinized by combination of enzymatic and the Sevage method, followed by dialysis (MW cut off 3500) with ultrapure water for 72 h. The crude polysaccharide preparation was obtained by freeze-drying. Crude PFLP was adsorbed onto DEAE-52 cellulose and washed off by eluent, then the PFLP was purified by Sephadex G-100 column (25 × 400 mm), following a previously reported method (Cheng *et al.*, 2013).

Determination of Molecular Weight

The mean molecular weight of PFLP was measured by the HPSEC method (Li *et al.*, 2014). Samples were dissolved into the mobile phase, centrifuged at 10000 rpm for 3 min and the supernatant collected for detection. The following conditions were used: 0.7% Na_2SO_4 mobile phase with a flow rate of 0.5 mL min^{-1}, the test solution of sample was 6 mg mL^{-1} and the volume of sample was 20 μL. Then according to the correction curves generated by a set of Dextran T-series standards with known molecular weights (Dextran T10, T40, T70 and T500), the molecular weight was estimated by GPC (Millennium 32 version).

Monosaccharide Composition Analysis

Compositional analysis of the sample was performed by the alditol acetate method (Dahlman *et al.*, 2000) with some alterations. The sample was treated with 2 mol L^{-1} trifluoroacetic acid and kept at 120ºC for 3.5 h to hydrolyze polysaccharides, which were then mixed with $NaBH_4$ in distilled water for 1.5 h. Pyridine was used as the catalyst and acetic anhydride as substrate. Standards including Glc, Gal, Rha, Ara, Xyl and Man, were also treated in parallel.

Congo Red Analysis

To determine the triple helical structures of PFLP, PFLP was diluted to 2 mg mL^{-1} in distilled water and 2.5 mL of the sample solution was added to varying concentrations of Congo red solution (0.0, 0.05, 0.10, 0.15, 0.2, 0.30, 0.35, 0.40, 0.45 and 0.50 mol L^{-1}). Absorbance from 200 nm to 800 nm was recorded and compared with a distilled water control (Yang *et al.*, 2012).

Fourier-transform Infrared Spectra Analysis

The FT-IR spectrum of PFLP was recorded with a FT-IR spectrometer (IRPrestige-21, Shimadzu, Japan) in the wave number range 4000–400 cm^{-1} by the potassium bromide disc method.

Smith Degradation and Periodate Oxidation

Periodate oxidation and Smith degradation of the sample was performed by the reported method (Li *et al.*, 2010). PFLP (10.0 mg) was incubated with 0.015 mol L^{-1} sodium metaperiodate (30 mL) at 4ºC in the dark, and the absorption at 223 nm was monitored. After 120 h later, the sample was mixed with ethylene glycol (0.2 mL) and stirred for 30 min, and the consumption of $NaIO_4$ was then measured. After further treatment, the hydrolysate of PFLP was analyzed by GC.

Partial Hydrolysis

PFLP was partly hydrolyzed by adjusting the pH of the solution (20 mL) to 2.0 with trifluoroacetic acid and incubating at 110ºC for 5 h. The precipitate was collected by centrifugation at 4000 rpm for 10 min and then monosaccharide composition was analyzed by GC. The supernatant was placed in a dialysis bag (*MW* cut off 3500) and dialyzed against H_2O for 48 h. Subsequently, the liquor in a bag and out of the bag were processed and analyzed *via* GC.

Methylation Analysis

Two milligrams of PFLP were methylated three times according to a previously reported method (Cui *et al.*, 1996). Following methylation, the OH band from 3200 cm^{-1} to 3700 cm^{-1} had disappeared compared to the initial IR spectrum without methylation, showing that PFLP was

completely methylated. The methylation of PFLP was analyzed by GC-MS according to the protocol of Needs (Cui *et al.*, 1996).

NMR Spectroscopy Analysis

PFLP was dissolved in deuterium oxide (D_2O) at a concentration of 3% for 3 h and then freeze-dried. This process was repeated 3 times then the ^1H spectra were read on a NMR spectrometer (500 MHz, ^1H) (VNMRS600, Agilent).

Antioxidant Activity Analysis

The reducing power of PFLP was measured according to a reported method (Dang *et al.*, 2013). A PFLP solution (1 mL) was mixed with phosphate buffer (2.5 mL), and aqueous potassium ferricyanide was added to a final concentration of 1% and incubated for 20 min at 50ºC. Samples were then quickly chilled, and 1.0 mL of 10% TCA was added. After centrifugation at 5000 rpm for 15 min, the supernatant (2.5 mL) was mixed with $FeCl_3$ (0.1%, 0.5 mL) and double distilled water (2.5 mL), and left to incubate for 15 min at 25 ºC. Absorbance of the mixed solution at 700 nm was recorded and compared to the absorbance of acid (Vc).

The Fe^{2+} chelating activity of PFLP was determined by a previously reported method (Manivasagan *et al.*, 2013). The PFLP solution (1 mL) was mixed with 0.2 mL ferrozine solution (5 mmol L^{-1}) and double distilled water (2.75 mL), and then 0.05 mL of $FeCl_2$ (2 mmol L^{-1}) was added, and the mixture was incubated at 25ºC for 15 min. Absorbance of the solution at 562 nm was recorded and compared to an EDTA-2Na samples, as well as a double distilled water blank. Fe^{2+} chelating activity was determined by the equation shown below:

$$Fe^{2+}\ \text{chelating activity}\ (\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \text{ in which}$$

A_0 was the absorbance of the double distilled water,
A_1 was the absorbance of the solution with PFLP in reactive system and
A_2 was the absorbance of the solution with PFLP

The superoxide anion radical scavenging activity of PFLP was measured according to a reported method (Wang *et al.*, 2014). A PFLP solution (0.5 mL) was mixed with pyrogallol solution (0.5 mL, 25 mmol L^{-1}) and Tris-HCl solution (2 mL, 50 mmol L^{-1}) in order, then incubated for 5 min at 25ºC. Hydrochloric acid was then added to stop the reaction. The absorbance of the solution at 560 nm was recorded and compared with ascorbic acid (Vc) and a double distilled water blank. The scavenging activity of superoxide anion radical was determined by the equation shown below:

The scavenging activity of superoxide anion radical:

$$(\%) = (1 - \frac{A_1 - A_2}{A_0}) \times 100, \text{ in which}$$

A_0 was the absorbance of the double distilled water, A_1 was the absorbance of the solution with PFLP in reactive system and A_2 was the absorbance of the solution with PFLP after reactive system aborted

The hydroxyl radical scavenging activity of PFLP was defined by a previously reported method (Gao *et al.*, 2013). The sample (1 mL) was mixed with H_2O_2 (1 mL, 9 mmol L^{-1}), salicylic acid-ethanol (1 mL, 9 mmol L^{-1}) and $FeSO_4$ (1 mL, 9 mmol L^{-1}). The mixture was incubated for 30 min at 37ºC, and the absorbance at 510 nm was recorded and compared with that for ascorbic acid (Vc) and a double distilled water blank. The scavenging activity of hydroxyl radical was determined by the equation shown below:

$$\text{The scavenging activity of hydroxyl radical}\ (\%) = (1 - \frac{A_1 - A_2}{A_0}) \times 100, \text{ in which}$$

A_0 was the absorbance of the double distilled water, A_1 was the absorbance of the solution with PFLP in reactive system and A_2 was the absorbance of the solution with PFLP

DPPH radical scavenging activity of PFLP was measured according to a reported method (Sharma and Bhat, 2009). Two milliliters of the sample solution was mixed with DPPH (2.5 mL). The mixture was incubated for 30 min at 25ºC and the absorbance of the solution at 517 nm was recorded and compared to the absorbance of ascorbic acid (Vc) and the absorbance of double distilled water blank. The scavenging activity of DPPH radicals was determined by the equation shown below:

$$\text{The scavenging activity of DPPH radical}\ (\%) = (1 - \frac{A_1 - A_2}{A_0}) \times 100, \text{ in which}$$

A_0 was the absorbance of the double distilled water, A_1 was the absorbance of the solution with PFLP in reactive system and A_2 was the absorbance of the solution with PFLP

ATBS radical scavenging activity of PFLP was measured according to a reported method (Cheng *et al.*, 2013). The sample solution was mixed with ABTS solution (3 mL). The mixture was to incubated for 6 min at 25ºC, and the absorbance of the solution at 510 nm was recorded and compared with the absorbance of ascorbic acid (Vc) and the absorbance of double distilled water blank. The scavenging activity of ABTS radical was determined by the equation shown below:

$$\text{The scavenging activity of ATBS radical}\ (\%) = (1 - \frac{A_1 - A_2}{A_0}) \times 100, \text{ in which}$$

A_0 was the absorbance of the double distilled water, A_1 was the absorbance of the solution with PFLP in reactive system and A_2 was the absorbance of the solution with PFLP

Results

Structural Analysis

The homogeneity of the PFLP preparation was determined

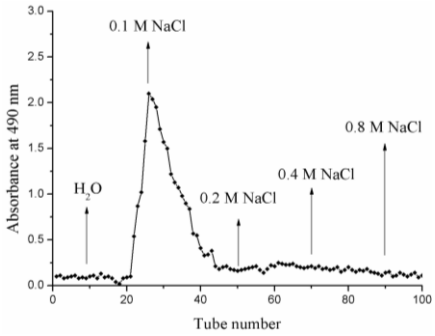

Fig. 1: Purification of PFLPs. Elution profile of PFLPs by DEAE-52 cellulose column chromatography

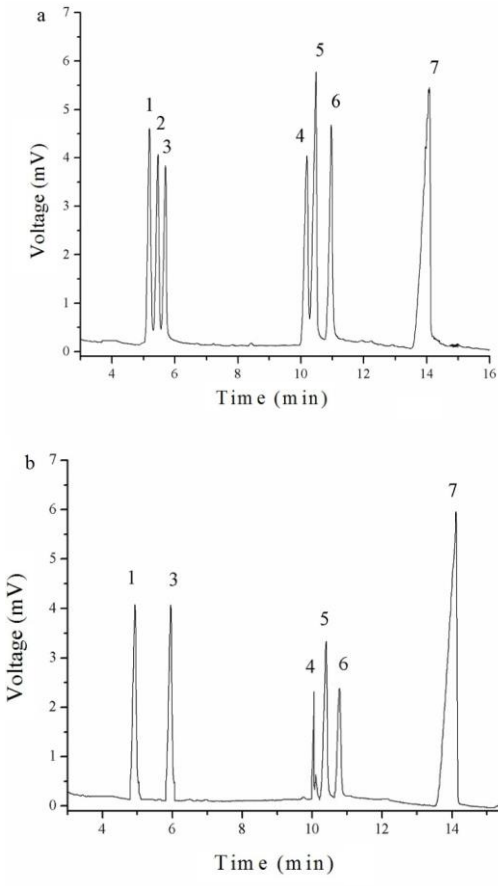

Fig. 2: Gas chromatograms of standard monosaccharide (**a**) and PFLPs (**b**). (1: Rhamnose; 2: Arabinose; 3: Xylose; 4: Mannose; 5: Glucose; 6: Galactose; 7: Inositol)

by a single, symmetric peak in the elution profile from a Sephadex G-100 column (Fig. 1). The molecular weight of PFLP was 10,100 Da, and the PFLP composed mainly of Rha, Xyl, Man, Glc, and Gal with a molar ratio of 1:1.26:0.85:12.4:4.31 (Fig. 2).

As shown in Fig. 3, the λ_{max} decreased rapidly when the concentration of NaOH was greater than 0.3 mol L^{-1} and the λ_{max} of PFLP also decreased. This means that a high order helix was dissociating. Afterwards, the λ_{max} reached a constant minimum value, which indicated that the helical structure of PFLP had completely dissociated. Congo red analysis showed that PFLP had a triple helical chain conformation.

As shown in Fig. 4, the FT-IR spectrum of PFLP revealed, a broad band at 3450 cm^{-1}, which was caused by O-H stretching vibrations of the polysaccharides. The band at 2954 cm^{-1} was due to C-H stretching oscillation of PFLP. The broad band at 1740 cm^{-1} was attributed to C=O stretching vibrations of -CHO, which indicated that the PFLP was a glycoconjugate containing protein. Specific bands at 1500 cm^{-1} and 1043 cm^{-1} were due to the C-O stretching oscillations of the pyranose ring. The band at 785 cm^{-1} was ascribed to β-type glycosidic linkages in the polysaccharide and bands at 870 cm^{-1} and 758 cm^{-1} were characteristic of β-D-glucan. Periodate oxidation of PFLP showed that 2.32 mmol of periodate was consumed and 1.02 mmol of formic acid was generated per sugar residue, giving periodate consumption at twice the rate of formic acid production. This indicated that PFLP possessed 1→2, (1→2, 6), 1→4 and 1→(4, 6)-linkages. Smith degradation of the periodate oxidized PFLP was analyzed by GC and revealed the presence of, glycerol and erythritol, which indicated that PFLP possessed 1→, 1→6, 1→2, 1→(2, 6), 1→4 and 1→(4, 6)-linkages. Glc was also found, which indicated that PFLP also possessed 1→3, 1→(3,6), and 1→(3, 4)-linkages.

The results of partial hydrolysis indicated the composition of the monosaccharide main and side chains. The PFLP-a indicated that the main chain of PFLP was composed mainly of Rha, Man, and Glc, and the PFLP-b indicated that the core region of the main chain was composed mainly of Rha, Glc, Man and Gal. The PFLP-c indicated that the terminal of the main and side chains composed mainly of Rha, Glc, Man and Gal (Table 1).

According to the retention time and the characteristic ion fragments in mass spectrometry, the linkage patterns of PFLP are shown as Table 2. The molar ratio of the monosaccharides was determined by the peak area. PFLP appeared to be a polysaccharide with branches, and its branch degree was pretty high. PFLP was composed mainly of (1→3)-linked Glu*p* (82.35%) as the main component with (1→3)-linked Man*p* (11.76%) and (1→3)-linked Rha*p* (5.89%) as minor components.

As shown in Fig. 5, the ^1H signal was mainly observed between δ 3.2–5.5 ppm. Glycosidic proton signals were seen between δ 3.5–4.5 ppm, while ^1H signals for β-H, γ-CH, γ-CH_2 and γ-CH_3 from amino acid residues of the protein were present at δ 1.12–3.66 ppm (Chauveau *et al.*, 1996). The 1H-NMR spectrum of PFLP showed seven anomeric protons (Fig. 5). Most of the anomeric protons were less than δ 5.0 ppm and they exhibited resonance signals between δ 5.4–4.3 ppm. This indicated that PFLP was composed mainly of β pyranoside bonds.

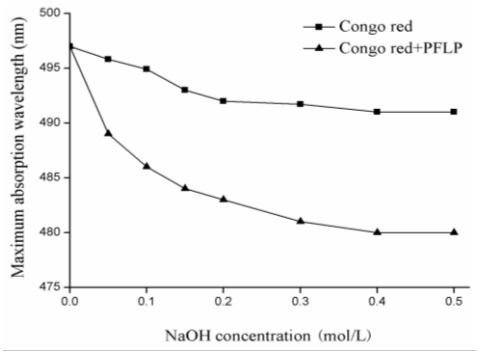

Fig. 3: The maximum absorption wavelength of Congo red at various concentrations of sodium hydroxide

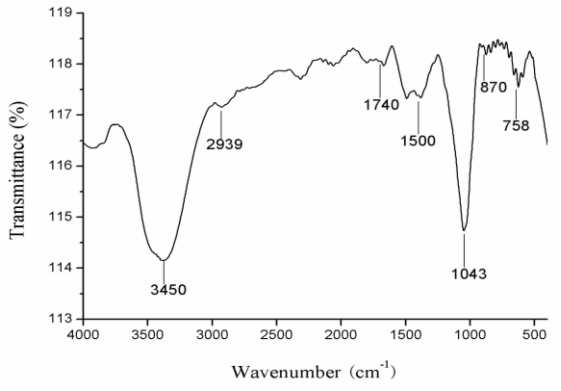

Fig. 4: FT-IR spectrum of PFLP

Based on the above-mentioned results, it can be concluded that PFLP was composed of a repeating unit with the main chain having a possible structure as follows:

PFLP: [→3)-β-D-Glu*p*-(1→3)-β-D-Man*p*-(1→2)-β-D-Rha*p*-(1→4)-β-D-Xyl*p*-[(1→3)-β-D-Glu*p*]$_5$-(1→3)-β-D-Glu*p*-1→]$_n$

Antioxidant Activity Analysis

The reducing power of PFLP is shown in Fig. 6a, and this reducing power increased with increasing concentrations of PFLP. When the concentration of PFLP was 5 mg mL^{-1}, its reducing power was 0.45, which was lower than that of V_C. It indicated the PFLP presented less reducing power.

The Fe^{2+} chelating activity of PFLP is shown in Fig. 6b, and this activity correlated well with increasing concentrations of PFLP. The chelating activity of PFLP was 31.38% at a concentration of 5 mg mL^{-1}. At the corresponding test concentrations, the chelating activity of PFLP was lower slightly than EDTA-2Na, which indicated that PFLP had lower Fe^{2+} chelating activity.

As shown in Fig. 6c, the superoxide anion radical scavenging activity of PFLP increased when the concentration of PFLP increased. The scavenging activity of PFLP was 55.38% at a concentration of 5 mg mL^{-1}. The IC50 value of PFLP was 4.68 mg mL^{-1} within the tested dosage range. However, its scavenging activity of superoxide anion radicals was relatively lower than that of Vc.

As shown in Fig. 6d, the hydroxyl radical scavenging activity of PFLP was dose-dependent. The scavenging activity of PFLP was 64.78% at 5 mg mL^{-1}. The IC50 value of PFLP was 4.41 mg mL^{-1} within the range of doses tested. This indicated that PFLP had good hydroxyl radical scavenging activity.

The DPPH radical scavenging activity of PFLP increased with increasing concentrations of PFLP increased (Fig. 6e). The scavenging activity of PFLP was 81.85% when the concentration of PFLP was 5 mg mL^{-1}. The IC50 values were 2.75 mg mL^{-1} within the range of doses tested. PFLP had strong DPPH radical scavenging activity.

The ABTS radical scavenging activity of PFLP is shown in Fig. 6f, which occurred in a concentration-dependent manner. The scavenging activity of PFLP was 94.26% at 5 mg mL^{-1}. The IC50 value was 1.47 mg mL^{-1} over the tested dosage range. The results indicated that PFLP had very good ABTS radical scavenging activity.

Table 1: Monosaccharide composition of PFLP fractions by partial acid hydrolysis. (a: The precipitate; b: In the dialysis bag; c: Outside the dialysis bag)

Parts	Molar ratio (%)				
	Rha	Xyl	Man	Glc	Gal
PFLP1-a	5.72 ± 0.08	-	8.54 ± 0.10	85.74 ± 0.44	-
PFLP1-b	4.97 ± 0.04	-	4.46 ± 0.07	71.13 ± 0.09	19.44 ± 0.11
PFLP1-c	-	-	15.72 ± 0.05	80.16 ± 0.52	4.12 ± 0.04

Table 2: Methylation analysis of PFLP-b

	No.	Component	Time (min)	Linkage	Molar ratio (%)
PFLP	1	2,5-Di-O-acetyl-3,4,6-tri-O-methyl-D-Man	6.031	1→	5.85
	2	5-O-acetyl-3,4-di-O-methyl-L-Rha	6.244	1→2	5.89
	3	3,5-Di-O-acetyl-2,4,6-tri-O-methyl-D-Glu	7.596	1→3	42.14
	4	3,5-Di-O-acetyl-2,4-di-O-methyl-D-Man	8.061	1→3	5.91
	5	3,5,6-Tri-O-acetyl-2,4-di-O-methyl-D-Glu	10.470	1→3	40.21

Discussion

Based on the analysis above, the PFLP obtained by hot water extraction had significant antioxidant activity. Because of its specific structure, the antioxidant activity of PFLP in different tests was not the same. In this study, specific attention should be paid to the relationship between the structure and antioxidant activity of PFLP.

Compared with complicated extraction methods involving acid hydrolysis and mechanical heat reflux with the need for expensive equipment and damage to the environmental, the hot water extraction method provides several advantages including lower investment costs, easy operation and low energy consumption. Meanwhile, the hot

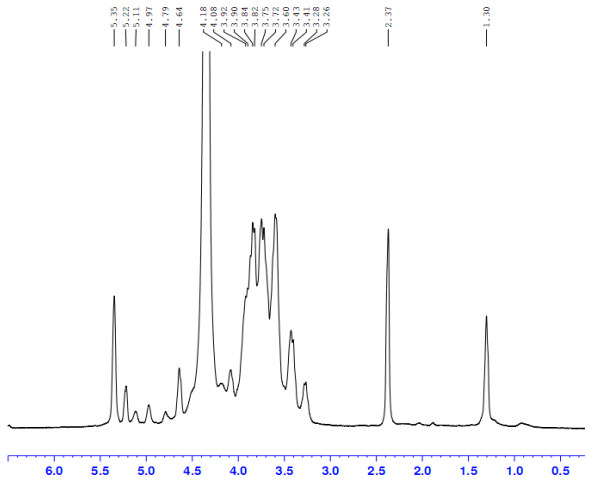

Fig. 5: ^{1}H-NMR spectrum of PFLP

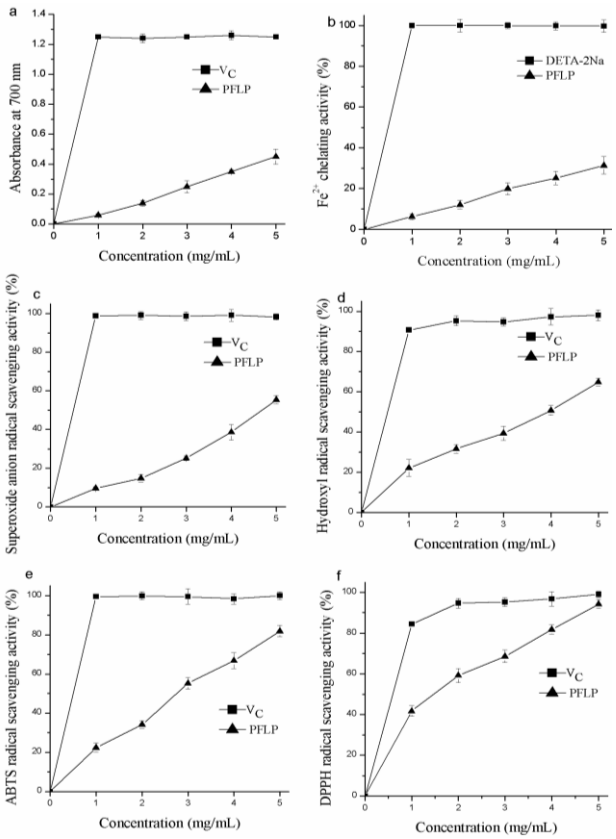

Fig. 6: Antioxidant activity tests of PFLP. (**a**) Reducing power. (**b**) Fe^{2+} chelating activity. (**c**) Superoxide anion scavenging activity. (**d**) Hydroxyl radical scavenging activity. (**e**) DPPH radical scavenging activity. (**f**) ABTS radical scavenging activity. Vc represents Vitamin C

water extraction yielded 7.82% more polysaccharides and 51.74% more carbohydrates than cold water extraction method, with 1.77% polysaccharides and 29.28% carbohydrates. Also, the polysaccharides prepared by ultrasonic-assisted extraction gave a yield of 2.21% and 47.59% carbohydrate (Fan *et al.*, 2017). Hence, hot water extraction seems to be a sensible and efficient method for isolating the polysaccharides from the fruiting body of *P. ferulae*.

Reactive oxygen species (ROS), are prooxidants, that include superoxide radicals, hydrogen peroxide, hydroxyl and DPPH, which are the products of normal metabolism (Delattre *et al.*, 2015). Excessive ROS can lead to irreversible damage to the human body (Li *et al.*, 2010) due to the fact that free radicals can cause severe damage to a broad range of macromolecules such as lipids, DNA, carbohydrates and proteins (Halliwell and Gutteridge, 1990), but antioxidants can protect the body by scavenging free radicals. Previous atudies have indicated that the reducing power, the chelating activity of Fe^{2+}, superoxide anion radical, hydroxyl radical, DPPH radical and ABTS radical can greatly reflect the antioxidant activity of a compound (Cheng *et al.*, 2013; Manivasagan *et al.*, 2013; Wang *et al.*, 2014).

In the current study, the difference in antioxidant activity between PFLP1 and PFLP2 could be explained as follows. First, the molecular weight of the polysaccharides could have an impact on their antioxidant effect, where a higher molecular weight could lead to better antioxidant activity. The antioxidant activity of PFLP2, including the reducing power, chelating activity of Fe^{2+}, superoxide anion radical, hydroxyl radical, DPPH radical and ABTS radical scavenging activity were slightly higher than that of PFLP1, possibly because of the similar monosaccharide composition between PFLP1 and PFLP2, but the higher molecular weight of PFLP2. Furthermore, the molar ratio of monosaccharides could also have influence on antioxidant activity, with different molar ratios of monosaccharides having different antioxidant activities. It should be noted that this coincides with the antioxidant activity of various polysaccharides. The polysaccharides extracted from *Polyporus umbellatus* sclerotia showed higher DPPH radical scavenging activity (nearly 60% at 1 mg mL^{-1}), superoxide anion radical scavenging activity (nearly 60% at 1 mg mL^{-1}) and hydroxyl radical scavenging activity (nearly 52% at 1 mg mL^{-1}), which has a similar β-linked D-glucan backbone but with a higher molecular weight of 1.44×10^4 Da (He *et al.*, 2016). Importantly, the glycosidic bond type and the complexity of polysaccharides may also influence antioxidant activity. The Se-polysaccharide obtained from Se-enriched *Grifola* frondosa had higher DPPH radical scavenging activity (nearly 40% at 1 mg mL^{-1}) and with a backbone consisting of α-linked D-Glcp and a molecular weight of 4.13×10^6 Da (Li *et al.*, 2017), the antioxidant activity could be due to the different configurations of the monosaccharide composition. At present, our study on the structure-activity relationship of polysaccharides is not perfect, and more studies are needed to find the answer the questions posed here.

Conclusion

In this study, a polysaccharide PFLP with a triple helical structure was isolated and purified from the fruiting body of *P. ferulae*. PFLP is a D-glucan containing a β-(1→3)-linked backbone with branched β-(1→6)-linkage. Antioxidant activity testing showed that PFLP possessed high hydroxyl radical scavenging activity (64.78% at 5 mg mL^{-1}), DPPH scavenging activity (81.85% at 5 mg mL^{-1}) and ABTS scavenging activity (85.97% at 5 mg mL^{-1}), moderate superoxide radical scavenging activity, and low reducing power, and Fe^{2+} chelating activity. Therefore, PFLP could be used as a potential antioxidant. Further investigations must be performed to assess other relevant biological activities of PFLP in order to consider its possible utilization as a functional food ingredient.

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