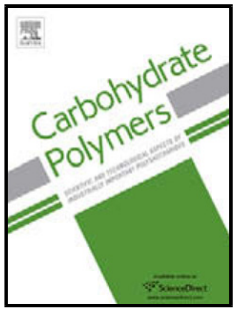

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Highlights

- A polysaccharide fraction (FMPS) was extracted from Huangshan floral mushroom for the first time.
- The primary structure and advanced structure of FMPS was studied.
- Investigated the antioxidant activity of FMPS by different manners.

Physicochemical properties and antioxidant activities of polysaccharide from floral mushroom cultivated in Huangshan Mountain

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Abstract:

In this paper, a polysaccharide fraction (FMPS) was purified from the floral mushroom cultivated in Huangshan Mountain for the first time. Physicochemical properties and antioxidant activities of FMPS were investigated. FMPS had an average molecular weight of 7.2×10^5 Da and was composed of glucose. On the basis of FT-IR, NMR and methylation analysis, the repeating unit of FMPS was established as (1→3)-linked *β*-*D*-glucopyranosyl backbone with 1-linked *β*-*D*-glucopyranosyl branches substituted at *O*-6 position of (1→3)-linked *β*-D-glucopyranosyl residues. The advanced structure studies indicated that FMPS was a triple-helical polysaccharide. The main hydrodynamic radius (R_h) of FMPS was 23.4 nm and it could form a stable system with water in 1.2×10^{-2} g/mL solutions. In addition, FMPS exhibited high DPPH radical scavenging activities (79.46% at 5 mg/mL) and hydroxyl radical scavenging activities (74.18% at 5 mg/mL), as well as Fe^{2+}chelating activities and ABTS radical scavenging activities to some extent.

Keywords: Floral mushroom; Polysaccharides; Physicochemical properties; Antioxidant activities.

1. Introduction

Mushrooms have been appreciated as important edible and medical resources (Villares, Mateo-Vivaracho, & Guillamón, 2012; Wani, Bodha, & Wani, 2010; Wasser, & Weis, 1999). A lot of mushrooms are known for their good flavor and texture, such as *lentinus edodes*, *flammulina velutipes* and *pleurotus ostreatus* (Xiong, 2009). In recent decades, the medicinal values of mushrooms attract people's attention greatly. Mushrooms are rich in many biologically active compounds of high medicinal values, such as lectins, terpenoids, polyphenolics, polysaccharides, ergosterols, and volatile organic compounds (Kalač, 2013). Especially, polysaccharide is a very important active ingredient of mushrooms. People have made much research of polysaccharides from edible mushrooms and find they exhibit antitumor (Zhang, Cui, Cheung, & Wang, 2007; Zhang, et al., 2013), antioxidant (Chen, Xie, Nie, Li, & Wang, 2008; Popov, et al., 2014; Xie, et al., 2010), and immunoregulation (Luo, Sun, Wu, & Yang, 2012; Yin, et al., 2012) activities. Thus, the deep study on polysaccharides from edible mushrooms together with its application is very meaningful.

A lot of polysaccharides have been extracted from edible mushrooms and many of them are *β*-linked glucans (Bohn, & BeMiller, 1995; Dai, et al., 2012; Villares, 2013; Wang, et al., 2009). Structures and functions of polysaccharides extracted from different edible mushrooms are different. It was reported that Zhuling polysaccharide, a water-soluble polysaccharide with (1→6, 1→4)-linked *β*-D-glucopyranosyl backbone, was a potent activator of B cells, macrophages and dendritic cells (Dai, et al., 2012). The polysaccharide isolated from the fruiting bodies of *Dictyophora indusiata*, consisting mainly of (1→3)-*β*-*D*-glucan with (1→6)-*β*-glucosyl side branches, had antitumor activities (Deng, et

al., 2013). The chain conformations of polysaccharides are closely related to their functions. For instance, the antitumor activity of lentinan with triple-helix chains is much higher than that in single flexible chains (Zhang, Li, Xu, & Zeng, 2005). It is necessary to study the structure of a novel polysaccharide and its conformations in aqueous solution.

Huangshan floral mushroom, hereinafter refer to as floral mushroom, grows in Huangshan Mountain, Anhui province, China (**Fig. 1A**). It is a rare species of edible mushrooms and looks like a flower with some grains in its top surface. Huangshan floral mushroom tastes delicious and has a high nutritional value. Though, polysaccharide is a kind of important active ingredients in floral mushroom. There are few reports on the polysaccharides from Huangshan floral mushroom. This limits the application of Huangshan floral mushroom. In this study, for the first time, a polysaccharide fraction was isolated from the fruiting body of floral mushroom (FMPS). The physicochemical properties and structural features of FMPS were elucidated by infrared spectroscopy, gas chromatography, gas chromatography-mass spectrometry, NMR and chemical methods. For further understanding of FMPS, the antioxidant activity of FMPS, including DPPH radical, hydroxyl radical, ABTS radical scavenging activities and Fe^{2+}chelating activities were tested. This study would provide meaningful data for the development of Huangshan floral mushroom.

2. Materials and methods

2.1 *Materials and reagents*

Dried fruiting bodies of floral mushroom were obtained from Huangshan city, Anhui province, China. Standard monosaccharides (D-glucose, D-mannose, D-xylose, L-galactose, L-rhamnose, and L-arabinose) and DMSO-d_6 were purchased from Sigma–Aldrich Co., Ltd.

(St. Louis, MO, USA). All other reagents were of analytical grade.

2.2 *Isolation and purification of polysaccharides*

The dried fruiting bodies of floral mushroom (**Fig. 1A**) were pulverized by a pulverizer and went through a 60-mesh screen before experiments. The powders（150 g） were defatted with petroleum ether and acetone successively by soxhlet extraction methord. The residues were dried at 50 ^{o}C and extracted twice with hot distilled water for 2 h at 100 ^{o}C. The rest of materials were further extracted with 5 L 0.05% $NaBH_4$/1.25 M NaOH solution twice at 25 ^{o}C for 2 h. The extracted solution was filtered with gauze to get the supernatants. The supernatants were neutralized by acetic acid and centrifuged to remove precipitation. 4 times volume of 95% ethanol was slowly added into the supernatants. The resulting mixture was centrifuged to get the precipitation after placed 24 h. The precipitation was dissolved in water again and removed proteins by Sevage methods. The resulting solution was decolorized with 30% H_2O_2 at 45 ^{o}C for 4h, dialyzed with tap water for 4 days and distilled water for 3 days. Dialysate was lyophilized to get the polysaccharide fraction FMPS (**Fig. 1B**). The yield was 4.7%.

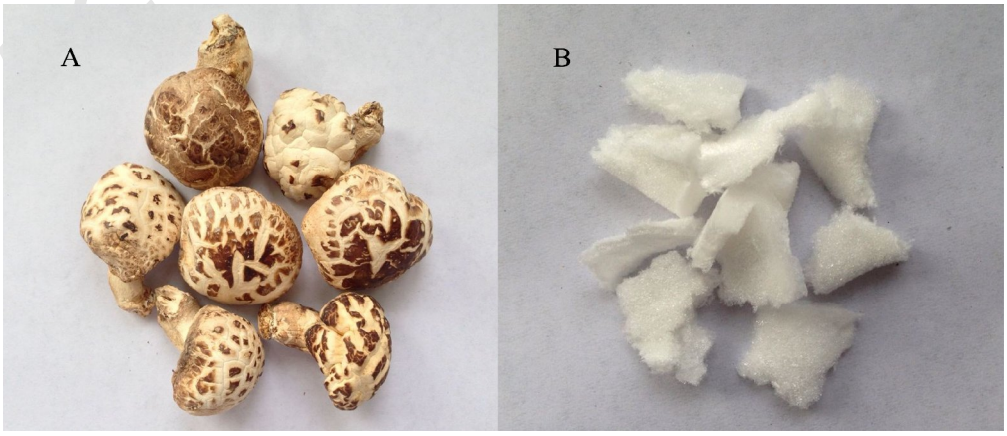

Fig. 1. Pictures of Huangshan floral mushroom (A) and the FMPS (B).

2.3 *Homogeneity and molecular weight*

The homogeneity and average molecular weight of FMPS were identified by high-performance gel-permeation chromatography (HPGPC) on a Waters E2695 HPLC system (Waters Corporation, USA) equipped with a Waters 2424 evaporative light scattering detector (ELSD). The Ultrahydrogel^{TM} 2000 column (7.8 mm×300 mm) and Ultrahydrogel^{TM} 500 column (7.8 mm×300 mm) were used as the separation medium, which were connected in series. The test conditions were as follows: sample concentration: 1mg/mL; mobile phase: ultrapure water; flow rate: 0.5 mL/min; injection volume: 20 μL. The calibration curve was created using a set of dextran standards.

2.4 *Fourier-transform infrared spectra (FT-IR) analysis*

FT-IR spectrum of FMPS was recorded on FT-IR spectrometer (Nicolet 5700, Thermo Nicolet, USA) in the wave-number range 4000–400 cm^{-1}. The testing method was potassium bromide (KBr) disc method (Xie et al., 2013).

2.5 *Monosaccharide composition analysis*

The monosaccharide composition of FMPS was analyzed by gas chromatography (GC). Sample (5 mg) was hydrolyzed by 4 mL 2 mol/L trifluoroacetic acid (TFA) at 120 ^{o}C for 4 h in a sealed glass tube. The residual TFA was removed using a rotary vacuum evaporator and the monosaccharides were reduced with 30 mg $NaBH_4$ for 3 h at room temperature after dissolved in distilled water. The reaction solution was neutralized with 25% CH_3COOH until there were no air bubbles. After removed the water phase, the residue was reacted with 3 mL acetic anhydride and 3 mL pyridine for 1 h. The reaction products were alditol acetate

derivatives and analyzed by GC. The operation method was reported in the previous study (Wang, Luo, & Zha, 2010).

2.6 *Methylation analysis*

The polysaccharide, FMPS (10 mg), was methylated using DMSO, sodium hydride and methyl iodide according to the Hakomori method (Needs & Selvendran, 1993). Complete methylation was confirmed by the disappearance of the OH band (3200–3700 cm^{-1}) in the IR spectrum. The methylated polysaccharide was depolymerized with 88% HCO_2H (3 mL) for 6 h at 100 ^oC in a sealed glass tube. After removing the residual formic acid, the polysaccharide was hydrolyzed by 4 mL 2 mol/L trifluoroacetic acid (TFA) at 120 ^oC for 4 h. The following treatment methods were the same to section 2.5. The resulting products were methylated alditol acetates and analyzed by GC–MS.

2.7 *NMR spectroscopy*

Sample (25 mg) was dried in P_2O_5 for 24 h, and then its ^{13}C NMR spectrum was recorded in DMSO-d_6 with NMR spectrometer (VNMRS600, Agilent) (Liu, Du, Wang, Zha, & Zhang, 2014).

2.8 *Advanced structure studies*

2.8.1 *Congo red analysis*

2 mL sample solution (2 mg/mL) was added to 2 mL Congo red solution (80 μmol/L) in a concentration gradient of NaOH solutions (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 mol/L). The absorbance was measured by UV-Vis spectrometer in the range of 200~800 nm. The distilled water without adding FMPS was served as the control. This method was reported in the previous literature (Liu, et al., 2014).

2.8.2 *Viscosity of FMPS in NaOH solutions*

A certain volume of distilled water and the NaOH solution were added to 2 mL sample solution (2 mg/mL). The final solution volume was 4 mL with a concentration gradient of NaOH (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 mol/L). The viscosity was tested by the rotational rheometer (DHR-3, TA, USA) with a 40 mm parallel plate.

2.8.3 *Dynamic light scattering*

Dried polysaccharide was dissolved in distilled water with the concentration of 1mg/mL. The solution was centrifuged to remove the impurities before testing. The test was performed on a dynamic light scattering instrument (MS800, Dynapro).

2.8.4 *Dynamic strain sweep*

The dependences of storage modulus (G') and loss modulus (G'') on the strain were investigated by the rotational rheometer (DHR-3, TA, USA) with a 60 mm parallel plate. The dynamic strain sweep measurements were repeated three times for 1.2×10^{-2} g/mL FMPS aqueous solution at 15 ^{o}C.

2.9 *Antioxidant activity test*

2.9.1 *DPPH radical scavenging activity*

The DPPH radical scavenging activities of FMPS were determined using the reported method (Chaiklahan, et al., 2013) with some modifications. The FMPS solutions were prepared with the concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL, respectively. 2.0 mL sample solution was mixed with 0.5 mL 100 μmol/L DPPH in methanol. Then the mixtures were shaken and allowed to equilibrate at room temperature for 30 min. The sample

absorbances were measured at 517 nm. In this study, the distilled water was used as the blank control and ascorbic acid (Vc) was served as positive control. The ability to scavenge the DPPH radical was calculated according the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_1 + A_2) / A_0 \times 100$$

Where, A_0, A_1 and A_2 were the absorbances of the control solution (water instead of FMPS), sample in reactive system and final absorbance of sample (methanol instead of DPPH), respectively.

2.9.2 *Hydroxyl radical scavenging activity*

The activity of FMPS to scavenge hydroxyl radical was determined according the reported method (Gao, Wang, Wang, & Wang, 2013). The FMPS solutions were prepared with the concentrations of 1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL, respectively. 1.0 mL sample solution was mixed with 1.0 mL $FeSO_4$ of 9 mmol/L, 1.0 mL salicylic acid of 9 mmol/L in ethanol and 0.05 mL H_2O_2 of 9 mmol/L. Then the mixtures were incubated at 37 ^oC for 30 min, the sample absorbances were measured at 510 nm. The distilled water was used as the blank control and ascorbic acid (Vc) was served as positive control. The scavenging activity of hydroxyl radical was calculated using the equation as follows:

$$\text{Hydroxyl radical scavenging activity (\%)} = (A_0 - A_1 + A_2) / A_0 \times 100$$

Where, A_0, A_1 and A_2 were the absorbances of the control solution (water instead of FMPS), sample in reactive system and final sample, respectively.

2.9.3 *ABTS radical scavenging activity*

The ABTS radical scavenging activity of FMPS was measured according to the reported method (Cheng, et al., 2013). The FMPS solutions were prepared with the concentrations of

1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL, respectively. 0.2 mL sample solution was mixed with 4.0 mL 7 mmol/L ABTS. Then the mixtures were incubated at room temperature for 30 min, the sample absorbances were measured at 734 nm. The distilled water was used as the blank control and ascorbic acid (Vc) was served as positive control. The scavenging activity of ABTS radical was calculated using the equation as follows:

ABTS radical scavenging activity (%)= $(A_0 - A_1 + A_2) / A_0 \times 100$

Where, A_0, A_1 and A_2 were the absorbances of the control solution (water instead of FMPS), sample in reactive system and final sample, respectively.

2.9.4 *Fe^{2+} chelating activity*

The Fe^{2+} chelating activity of FMPS was tested according to the previous method (Manivasagan, et al., 2013). 1 mL sample containing different concentrations of FMPS (1~5 mg) was mixed with deionized water (2.75 mL), $FeCl_2$ (0.05 mL 2 mM) and ferrozine (0.2 mL, 5 mM). The mixture was incubated for 10 min at room temperature and the absorbance was determined at 562 nm. The distilled water was used as the blank control and ethylene diamine tetraacetic acid disodium salt (EDTA-2Na) was served as positive control. The Fe^{2+} chelating activity was calculated according the following equation:

Fe^{2+} chelating activity (%)=$(A_0 - A_1 + A_2) / A_0 \times 100$

Where, A_0, A_1 and A_2 were the absorbance of the control solution (water instead of FMPS), sample in reactive system and sample, respectively.

3. Results and discussions

3.1 *Isolation, purification and composition analysis of FMPS*

A water-soluble polysaccharide FMPS was isolated and purified from the fruiting body

of floral mushroom cultivated in Huangshan Mountain. The homogeneity and average molecular mass of FMPS were identified by HPGPC and it showed a single and symmetrical peak (**Fig. 2A**) with an estimated molecular weight 7.2×10^5 Da based on dextran standards. The monosaccharide composition of FMPS was analyzed by GC (**Fig. 2B)**. As shown in Fig. 2B, FMPS was composed of glucose only based on the GC analysis of standard monosaccharide.

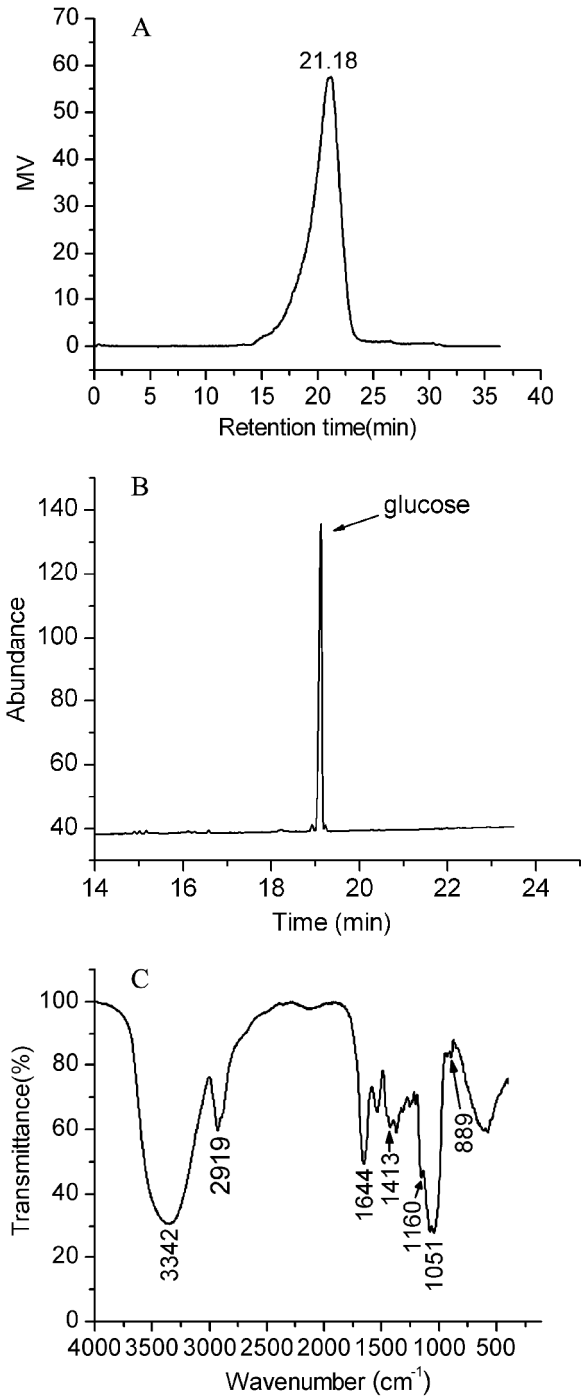

Fig. 2. (A) HPGPC chromatogram of FMPS; (B) GC chromatogram of monosaccharides composition of FMPS; (C) FT-IR spectrum of FMPS.

3.2 *FT-IR spectrum analysis*

The FT-IR spectrum of FMPS was showed in **Fig. 2C**. A typical broad peak at 3342 cm^{-1} was due to the stretching vibration of O-H in the sugar ring. The peaks at 1160 cm^{-1} and 1051cm^{-1} suggested the presence of C-O bonds in FMPS (Du, Liu, & Wang, 2015). The absorption band at 2919 cm^{-1} was due to the C-H stretching vibration (Suvakanta, Narsimha, Pulak, Joshabir, & Biswajit, 2014). A little absorption peak around 1413 cm^{-1} was attributed to the deformation vibration of C-H. The absorption peak at 889 cm^{-1} indicated a β-D-glucan existing in the polysaccharide (Wang, et al., 2009). No absorption peaks at 1730 cm^{-1} suggested that there were no uronic acids in FMPS (Zhang, Xiao, Deng, He, & Sun, 2012).

3.3 *Linkage types analysis*

The FMPS was methylated twice according to the Hakomori method (Needs & Selvendran, 1993). FT-IR spectrum of methylated polysaccharide was recorded on a FT-IR spectrometer and the result was shown in **Fig. 3A**. FT-IR spectrum indicated that the methylation was complete indicative of the absences of absorption peaks at 3342 cm^{-1}. The absorption peak of C-H stretching vibration at 2919 cm^{-1} had obvious enhancement due to the addition of methyl.

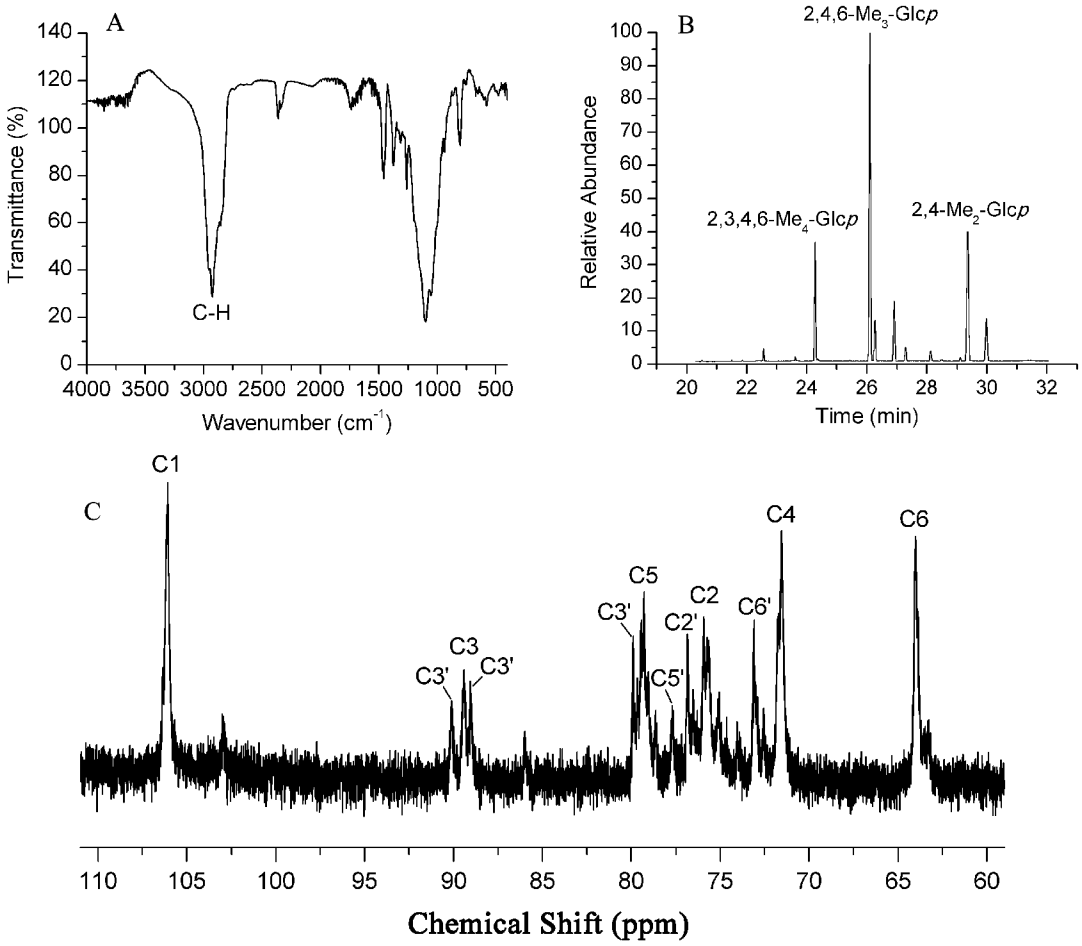

Fig. 3. (A) FT-IR spectrum of methylated FMPS; (B) Total ion chromatogram of FMPS by GC–MS; (C) ^{13}C NMR spectrum of FMPS in DMSO-d_6.

The methylated polysaccharides were converted into partially methylated alditol acetates and analyzed by GC-MS (**Fig. 3B**). The methylation analysis revealed mainly three partially methylated alditol acetates 1, 5-di-acetyl-2, 3, 4, 6-tetra-*O*-methyl glucitol, 1, 3, 5-tri-acetyl-2, 4, 6-tri-*O*-methyl glucitol, and 1, 3, 5, 6-tetra-acetyl-2, 4-di-*O*-methyl glucitol with a molar ratio of 1.50: 3.44: 1.45. And their corresponding peak times were 24.28 min, 26.10 min and 29.36 min, respectively (**Table 1**). The other absorption peaks in **Fig. 3B** were

attributed to solvent and other chemical reagents added during methylation. Accordingly, the following three glucosidic linkages existed in FMPS: non-reducing terminal glucosyl, (1→3)-linked glucosyl and (1→3, 6)-linked glucosyl. It showed that (1→3)-linked-glucosyl was the largest amount residue in FMPS and (1→3, 6)-linked-glucosyl was the branched residue, revealing that (1→3)-linked-glucosyl should be possible to form the backbone structure (Wang, et al., 2013). The relative molar ratio of (1→3)-linked-glucosyl and (1→3, 6)-linked-glucosyl was 2.29 indicating that average one branching point for each three or four residues of backbone. The residues of branch structure were 1-linked glucosyl residues.

Table 1. GC-MS data for methylation analysis of FMPS.

Retention time	Methylated sugars	Linkage types	Molar ratios	Mass fragments (m/z)
24.28	2,3,4,6-Me_4-Glc*p*	Terminal	1.50	45, 71,87,101,117,129,145,161,205
26.10	2,4,6-Me_3-Glc*p*	1,3-Linked-Glc*p*	3.44	45,58,71,87,97,101,117,129,161,173,233
29.36	2,4-Me_2-Glc*p*	1,3,6-Linked-Glc*p*	1.45	58,87,99,101,117,129,159,173,189,233

3.4 *NMR spectroscopy analysis*

The ^{13}C NMR spectrum of FMPS in DMSO-d_6 was shown in **Fig. 3C**. Compared with the data on similarity substituted sugar residues in previous literatures (Wang, et al., 2009; Corsaro, et al., 2005; Zhang, Zhang, Dong, Guo, & Song, 2001), the chemical shifts at 106.1 (C1), 75.9 (C2), 89.4 (C3), 71.6 (C4), 79.3 (C5) and 64.0 (C6) *ppm* were assigned to the carbon atoms of the (1→3)-*β*-D-glucan backbone. The peaks at C3′ (90.1, 89.1, 79.9), C5′ (77.6), C2′ (76.8), and C6′ (73.1) were resulted from branching effect. The structure features

of FMPS were similar to those reported polysaccharides such as JQPs (Liu, et al., 2014) and PD3 (Wang, et al., 2009), which had a (1→3)-*β*-D-glucan backbone with (1→6)-*β*-glucosyl side chains.

Combining the ^{13}C NMR spectrum and methylation analysis of FMPS, it was concluded that the FMPS had (1→3)-*β*-*D*-glucopyranose backbone, and *O*-6 position of (1→3)-linked *β*-D-glucopyranose was substituted by 1-linked-*β*-D-glucopyranosyl branches. There was average one branching point for each three or four residues of backbone.

3.5 *Advanced structure of FMPS*

Congo red could form special complexes with polysaccharides possessed an ordered three-dimensional structure, generally triple-helical conformation in solution, and the maximum absorption wavelength (λ_{max}) in spectral scan would shift (Nitschke, et al., 2011). The complex was stabilized by hydrophobic interactions or strong hydrogen bonds between the polysaccharide and the dye molecule (Palacios, García-Lafuente, Guillamón, & Villares, 2012). The interactions of FMPS with Congo red were shown in **Fig. 4A**. The λ_{max} increased to a maximum with the C_{NaOH} increased initially and decreased sharply when the C_{NaOH} researched 0.2mol/L, indicating that FMPS displayed a triple-helical structure and which was destroyed in high concentrate NaOH solution (Du, Liu, & Wang, 2015).

Moreover, the viscosities of FMPS in a series of NaOH solutions were tested at the shear rate of 100 s^{-1} by the rotational rheometer on the temperature 25 ^{o}C. The result was shown in **Fig. 4B**. In order to eliminate the influence of solvent, the viscosities of NaOH solutions were also tested. The viscosities of NaOH solutions had no obvious change in the selected concentration range. The viscosities of FMPS increased when the concentration of

NaOH reached 0.05 mg/mL. When the concentration of NaOH more than 0.05 mg/mL, the viscosities of FMPS reduced gradually with the increasing of NaOH concentration and reached a steady value. The reason that the viscosities of FMPS increased in low concentration of NaOH solution was due to that part of hydrogen bonds in the triple-helical chains was destroyed and the extension of the molecular chain was intensified. With the further increasing of concentration of NaOH solutions, the conformation of FMPS had transformed from triple-helical chain into flexible chain. This conformation transformation made the molecular chains fully collapse and they were less contact with each other, which resulted in the decreasing of viscosities of FMPS. Similar phenomenon was observed in *Schizophyllan* (Zhou, Guo, Cai, & Li, 2005). It had been also reported that the (1→3)-*β*-D-glucan from *Poriacocos* sclerotium could form flexible chain conformation in the alkaline aqueous solution (Chen, Xu, Zhang, & Kennedy, 2009).

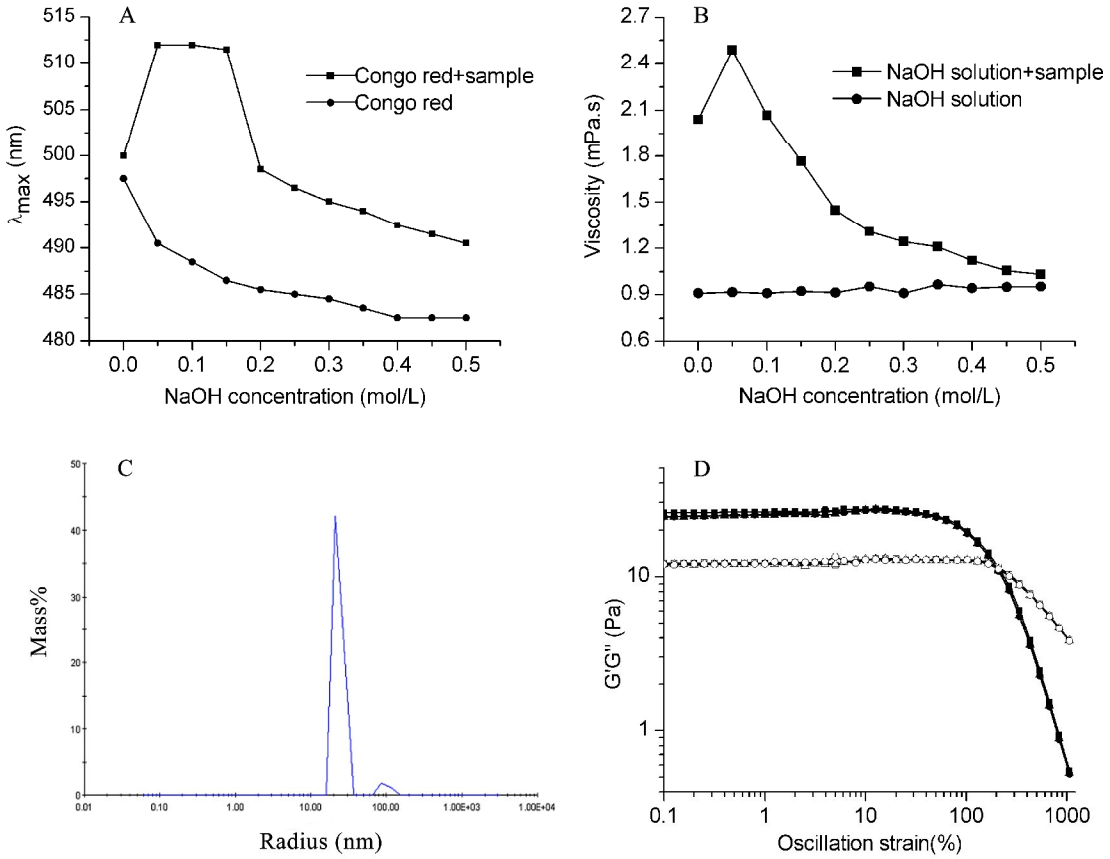

Fig. 4. (A) The maximum absorption wavelength of Congo red with FMPS and Congo red in various concentrations of sodium hydroxide solution; (B) The viscosities of FMPS in various concentrations of sodium hydroxide solution and the viscosities of sodium hydroxide solutions; (C) Dynamic light scattering spectrum of FMPS; (D) Strain dependence of shear storage modulus G' (solid symbols) and loss modulus G'' (open symbols) determined by dynamic strain sweep measurement for FMPS solutions with concentration of 1.2×10^{-2} g/mL at 15 ^{o}C.

For further understanding of the polysaccharide chain morphology in aqueous solution, 1 mg/mL FMPS solution was tested by dynamic light scattering (**Fig. 4C**). The results indicated that the hydrodynamic radius (R_h) of FMPS was composed of two parts, 23.4 nm

and 96.5 nm. The percentage of them was 95.7% and 4.3%, respectively. This suggested that FMPS mainly presented as single triple-helical chains in dilute solution. R_h of 96.5 nm maybe resulted from a little aggregation (Zhang, Li, & Zhang, 2010). The results of dynamic light scattering showed that the M_W of FMPS was 6.18 $\times 10^5$ Da. It was very close to the results of HPGPC.

Lentinan, which presenting triple-helical conformation in aqueous solution, could form a stable system with water in appropriate condition. This system was stable in a certain strain range and had good reproducibility even it was destroyed (Zhang, Xu, & Zhang, 2008). Since FMPS had a triple-helical structure, dynamic strain sweep was repeated three times for 1.2 × 10^{-2} g/mL FMPS aqueous solution at 15 ^{o}C. The second and third sweep began immediately after the end of the former sweep. Results of the above experiments were shown in **Fig. 4D**. As shown in **Fig. 4D**, G' and G'' were relatively stable before the strain research 100%. When strain was more than 100%, the values of G' and G'' reduced sharply, indicating that the structure of FMPS in solution had been destroyed. Moreover, three curves coincided very well, suggesting it was able to immediately return to original state even the structure was widespread destruction in previous sweep. The results revealed that FMPS could form a stable system with water under appropriate condition and the FMPS/water system had good reproducibility. The property of FMPS was meaningful for its application in food industry.

3.6 *Antioxidant activity analysis*

In this study, the antioxidant activities of FMPS were measured (**Fig. 5**). The DPPH free radical is a stable free radical, which has been widely used to determine the free radical scavenging abilities of antioxidants (Xie, et al., 2012). As shown in **Fig. 5A**, the DPPH

radical scavenging activities increased with the increase of concentration from 1 to 5 mg/mL. When the concentration increased to 5 mg/mL, the scavenging ratio of FMPS and Vc were 79.46% and 95.75%, respectively. The IC50 value of FMPS was 1.97 mg/mL within the concentration of test range. The results showed that FMPS had a strong DPPH radical scavenging ability. However, it was lower than Vc at the same concentration.

Hydroxyl radical is considered as one of the most reactive oxygen radicals, which can easily cross cell membranes, readily react with most biomolecules and lead to tissue damage or cell death (Gao, et al., 2013). **Fig. 5B** showed that the hydroxyl radical scavenging ability of FMPS had concentration dependence. It increased to 74.18% when the concentration increased to 5mg/mL. Within the test concentration range, the IC50 value of FMPS was 2.58 mg/mL. It showed that FMPS had a strong hydroxyl radical scavenging ability.

Previous study suggested that there were two type of mechanism on hydroxyl radical. One suppressed the generation of the hydroxyl radical and the other scavenged the hydroxyl radicals generated (Qi, et al., 2005). The former referred to that the polysaccharide ligated to the metal ions which reacted with H_2O_2 to give a hydroxyl radical (Qi, et al., 2005). It indicated that the polysaccharide may have Fe^{2+}chelating activity. Thus, the Fe^{2+}chelating activity of FMPS was tested.

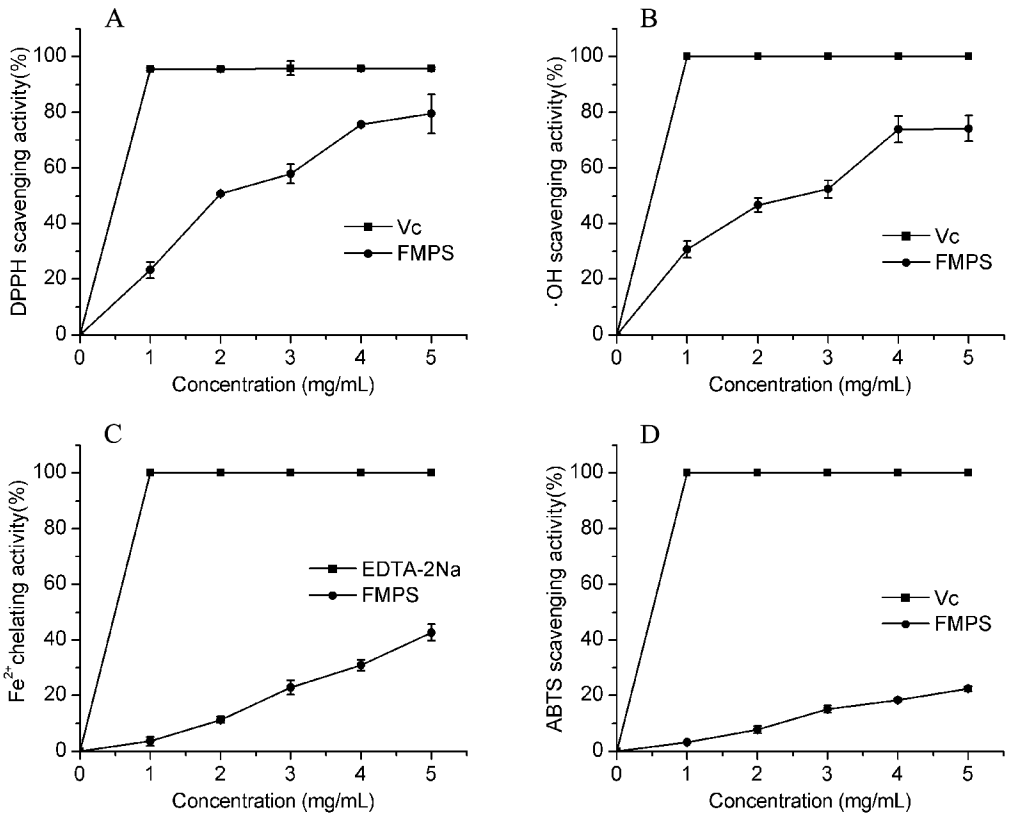

Fig. 5. Antioxidant activity tests of FMPS. (A) DPPH radical scavenging activity; (B) Hydroxyl radical scavenging activity; (C) Fe^{2+}chelating activity; (D) ABTS radical scavenging activity.

Iron was an essential microelement for organisms, but overloaded iron would catalyze the oxidation of protein, lipid and other components (Liu, et al., 2014).The Fe^{2+}chelating activity of FMPS was shown in **Fig. 5C**. The results showed that the chelating activity had concentration dependence. The value was only 3.59% at the concentration of 1mg/mL, but it had a sharp increase with the increase of concentration. The chelating activity increased to 42.68% when the concentration was 5 mg/mL. These results indicated that the FMPS had a moderate Fe^{2+}chelating activity at the concentration of 1-5 mg/mL.

The test of ABTS radical scavenging activity is also a widely used method to measure the antioxidant activity of chemical components (Re, et al., 1999; Shao, Chen, & Sun, 2013). In this study, the ABTS radical scavenging activity of FMPS was tested and the results were shown in **Fig. 5D**. The scavenging effect of FMPS on ABTS radical increased as the concentration increasing. The scavenging ratio of FMPS was 22.51% at the concentration of 5 mg/mL. The result showed that FMPS exhibited some ABTS radical scavenging activity.

In this study, the purified polysaccharide (FMPS) showed different antioxidant activities in various reactive systems, which may be due to its specific structures. Future research should focus on the relationship between the antioxidant mechanism and the structure of the polysaccharide. Furthermore, the antioxidant activity of the FMPS *in vivo* should be also investigated.

4. Conclusion

In this study, a polysaccharide fraction FMPS was isolated and purified from the fruiting body of floral mushroom cultivated in Huangshan Mountain, a precious edible mushroom. FMPS had an average molecular weight of 7.2×10^5 Da and was composed of glucose. Structural analysis revealed that FMPS had (1→3)-*β*-*D*-glucopyranose backbone and *O*-6 position of (1→3)-linked-*β*-D-glucopyranosyl residues was substituted by 1-linked-*β*-D-glucopyranosyl branches. There was average one branching point for three or four residues of backbone. Congo red analysis showed that the FMPS was a triple-helical polysaccharide. Its viscosity increased slightly and then reduced with the increase of NaOH concentration. Dynamic light scattering test indicated that the hydrodynamic radius (R_h) of FMPS was mainly 23.4 nm and it presented as single triple-helical chains in 1 mg/mL solutions. Dynamic strain sweep showed that FMPS could form a stable system with water in 1.2×10^{-2} g/mL solutions. The antioxidant activity tests *in vitro* indicated that FMPS had high DPPH radical scavenging activities (79.46% at 5 mg/mL), hydroxyl radical scavenging activities (74.18% at 5 mg/mL), some Fe^{2+}chelating activity and ABTS radical scavenging activity. These results indicated that FMPS could be used as a natural antioxidant. Future research should focus on the relationship between the antioxidant mechanism and the structure of the polysaccharide FMPS.

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