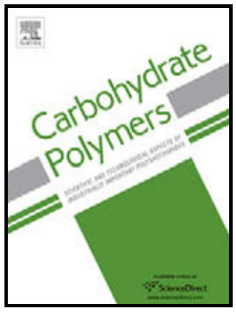

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Purification, characterization, and bioactivities of a polysaccharide from mycelial fermentation of *Bjerkandera fumosa*

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Highlights

- A homogeneous polysaccharide was purified from *B. fumosa.*
- The chemical characterizations of the purified polysaccharide (designated as DBFM3) were performed by HPLC, FT-IR, NMR.
- Antioxidant and immunoregulatory activities in vitro of purified polysaccharide from *B. fumosa* were studied.
- DBFM3 presents a promising natural source of antioxidant and immunoregulatory agents.

Abstract

In this work, a novel polysaccharide (named DBFM3) was isolated from mycelia of *Bjerkandera fumosa* by DEAE-32 and Sepharose CL-6B column chromatography. High-performance gel permeation chromatography showed that DBFM3 was homogeneous, with an average molecular weight of 1.8×10^5 Da. Structural characteristics of the purified fraction were investigated by high-performance liquid chromatography (HPLC), FT-IR, and NMR. HPLC analysis indicated that DBFM3 was composed of mannose, galacturonic acid, and galactose at molar ratios of 1:18.16:0.702. Spectral analysis suggested that DBFM3 had (1→6), (1→3,6), (1→3) linkages and pyranose conformation. Antioxidant assay in vitro showed that DBFM3 exhibited 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activity, and has protective effect against DNA damage and damage to SH-SY5Y cells induced by H_2O_2. Immunological tests indicated that DBFM3 significantly increased

lymphocyte proliferation in vitro. Furthermore, DBFM3 increased the proliferation of lymphocytes in the presence of concanavalin A or lipopolysaccharide as mitogen.

Keywords

Bjerkandera fumosa; Polysaccharide; Purification; Bioactivities

Chemical compounds studied in this article:

DPPH (PubChem CID: 2735032)

Ascorbic acid (PubChem CID: 54670067)

MTT (PubChem CID: 64965)

Hydrogen peroxide (PubChem CID: 784)

Rutin (PubChem CID: 5280805)

Galacturonic Acid (PubChem CID: 439215)

D-galactose (PubChem CID: 6036)

D-glucose (PubChem CID: 79025)

1. Introduction

A number of edible mushrooms have medicinal properties and have gained attention as functional health promoters due to their biochemical composition (Zhang, Cui, & Cheung, 2007). Polysaccharides are important bioactive constituents of medicinal fungi and possess numerous bioactivities, such as immunomodulation, anticancer, antioxidant, and hypoglycemic activities (Kardosova & Machova, 2006;

Liuet al., 2016; Schepetkin & Quinn, 2006). Polysaccharides are known for their roles as structural elements of cell walls and have significant effects on multi-cytokine inducers in cell-cell communication, for instance, in inducing gene expression of various immunomodulatory cytokines (Ooi & Liu, 2000). Several studies have demonstrated that the activities of polysaccharides are associated with the chemical composition and structural characteristics (Methacanon, Madla, & Kirtikara, 2005).

Bjerkandera fumosa (Pers.) P. Karst, which is widely distributed in China including Jilin, Hebei, Qinghai, and Liaoning provinces, is an annual wood-decaying fungus used in traditional Chinese medicine. Water extraction of the fruiting body is considered to be effective for treating uterine carcinoma and enhancing immunity. However, the active components of *B. fumosa* have not been reported. Recently, many natural polysaccharides and polysaccharide-protein complexes, such as lentinans from *Lentinus edodes* (Xu, Yan, & Tang, 2014) and PSP from *Coriolus versicolor* (Cui & Chisti, 2003), have been studied as immune modifiers and drugs for cancer therapies (Giavasis, 2014; Stachowiak & Reguła, 2012; Yang & Zhang, 2009). Therefore, the properties of polysaccharides from *B. fumosa* should be studied to better understand the activity of the fungus.

In the present study, a polysaccharide (named DBFM3) was purified from *B. fumosa* and characterized by chemical methods. The antioxidant effect of DBFM3 was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging assay, as well as its protective effect on DNA damage and SH–SY5Y cells damage induced by H_2O_2 was studied. In addition, the immunomodulatory effects on

splenocyte proliferation were investigated. This paper is the first to report the identification and biological activities of polysaccharides from *B. fumosa*.

2. Materials and methods

2.1. Materials

B. fumosa was purchased from the China Forestry Culture Collection Center (CFCC, Beijing, China). DEAE-32 cellulose and Sepharose CL-6B were purchased from Amersham (Sweden). 3-(4,5-Dimethyl tiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT), bovine serum albumin (BSA), DPPH, lipopolysaccharide (LPS), concanavalin A (ConA), and glucan standards were purchased from Sigma Co. (USA). The solvents and other chemicals used were analytical grade.

2.2. Mycelia and polysaccharide prescription

B. fumosa was maintained on potato dextrose agar slants. A 10 mm^2 portion of the agar with mycelia was inoculated in the seed medium (0.1 g/L $CaCl_2$, 1 g/L KH_2PO_4, 1.5 g/L $MgSO_4$, 2 g/L yeast extract, 3 g/L peptone, and 20 g/L glucose) for 5 days at 28°C and in a rotary shaker at 150 rpm. Then, the seed medium was added to a 500 mL flask containing 200 mL of fermentation medium and inoculated at a rate of 10% (v/v). The fermentation medium contained 0.5 g/L $MgSO_4$, 0.05 g/L $FeSO_4$, 1 g/L KH_2PO_4, 0.02 g/L $CuSO_4$, 0.4 g/L K_2HPO_4, 0.01 g/L $ZnSO_4$, 0.01g/L $CoCl_2$, 0.08 g/L $MnSO_4$, 3 g/L peptone, and 53 g/L corn powder. The medium was cultured in a rotary shaker (150 rpm) at 28 °C for 8 days. Mycelia of *B. fumosa* were filtered with gauze after cultivation.

The cultured mycelia were washed with distilled water two times, and extracted with boiling water for 2 h. This process was repeated three times. All water extracts were combined, concentrated, and precipitated by adding four volumes of 95% ethanol under vigorous stirring and incubated at 4 ℃ overnight. The precipitate was collected by centrifugation (4000 rpm for 15 min) and successively washed with absolute ethanol and ether. Finally, the crude mycelium polysaccharide (BFM) was lyophilized to obtain a taupe white powder.

BFM was dissolved in distilled water (5%), frozen-thawed, and centrifuged at 4 ℃ until no insoluble materials were present, and then deproteinized by a combination of pronase and Sevag methods (Xu, Zhou, & Hao, 2015). The deproteinized polysaccharide (DBFM) was passed through a DEAE-32 cellulose (2.6 cm×20 cm) column, and eluted first with distilled water, then with a linear gradient of NaCl from 0 M to 1.0 M at a flow rate of 1 mL/min. The eluate was collected automatically, and the polysaccharide content was monitored by the phenol-sulfuric acid method. The eluted samples were collected, concentrated, and further purified by gel filtration chromatography on Sepharose CL-6B column (2.5 cm×90 cm) using 0.9% NaCl as the eluant at a flow rate of 0.15 mL/min. The purified fractions were collected, treated with four volumes of ethanol, centrifuged, and then lyophilized.

2.3. Molecular weight determination

The homogeneity and molecular weight of DBFM3 were determined by high-performance gel permeation chromatography (HPGLC), which was performed on a Shimadzu HPLC system with a TSK-G3000PWXL column (7.8 mm×30.0 cm)

and a Shimadzu RID-10A detector. The sample solution (2 mg/mL) was injected and run with 0.2 mol/L NaCl as mobile phase at a flow rate of 0.6 mL/min.

2.4. Chemical components and monosaccharide composition

Polysaccharide content was determined by phenol-sulfuric acid spectrophotometric assay with D-glucose as a standard (Masuko et al., 2005). The uronic acid content was evaluated aby the m-hydroxydiphenyl colorimetric method with *D*-glucuronic acid as a standard, in which neutral sugars don't interfere (Filisetti & Carpita, 1991). The Bradford method was used to determine the protein content of polysaccharides using BSA as a standard (Bradford, 1976).

Monosaccharide composition of DBFM3 was determined as follows. The polysaccharide (2 mg) was hydrolyzed with 2 M trifluoroacetic acid at 120 °C for 2 h in a sealed-tube. The released monosaccharides were derivatized by 1-phenyl-3-methyl-5-pyrazolone and analyzed on a DIKMA Inertsil ODS-3 column (4.6 mm×150 mm) connected to a Shimadzu HPLC system (LC-10ATvp pump and SPD-10AVD UV-vis detector).

2.5. UV, FT–IR, and NMR analyses

Ultraviolet and visible (UV-vis) absorption spectra were recorded using a Shimadzu MPS-2000 spectrophotometer (200-700 nm).

Purified sample (2 mg) was mixed with KBr powder and pressed into a 1 mm pellet for IR analysis. The FT-IR spectrum was recorded on SPECORDIR in the range of 4000-500 cm^{-1}.

The ^{13}C-NMR and ^{1}H-NMR spectroscopic analyses were conducted on a Bruker

-DRX Avance 600 MHz (Germany) spectrometer at 150 MHz and 500 MHz at 28°C, respectively. The sample (20 mg) was dissolved in D_2O (1 mL, 99.8%) with overnight stirring at room temperature. Two-dimensional (2D) correlation spectroscopy (COSY) techniques were used for general assignments of DBFM3.

2.6. Antioxidant activity test in vitro

2.6.1. DPPH scavenging assay

DPPH radical scavenging activity was estimated according to the literature with minor modifications (Sharm & Bhat, 2009). The reaction mixture (OD_1) contained 2 mL of DPPH (0.1 M in methanol) and 2 mL of different concentrations of the polysaccharide solution (0.5, 1, 2, 3, 4, and 5 mg/mL). The reaction solution was shaken vigorously and incubated in the dark at room temperature for 30 min, and the absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as a positive control. OD_0 was measured using distilled water (instead of sample solution) as blank, and OD_2 was measured using methanol (instead of DPPH solution) as control. The DPPH antioxidant activity was calculated using the following formula:

$$\text{Scavenging rate}(\%) = \frac{OD_0 - (OD_1 - OD_2)}{OD_0} \times 100\%$$

2.6.2. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of purified polysaccharide was determined as described previously in the literature (Li, Huang, & Lu, 2011). The reaction mixture (A_x) contained 1 mL of samples at different concentrations (0.5, 1, 2, 3, 4, and 5 mg/mL), 1 mL of 9 mM salicylic acid-ethanol solution , 1 mL of 9 mM $FeSO_4$ solution, and 1 mL of 8.8 mM H_2O_2. After incubating at 37 °C for 1 h, the

mixture was centrifuged at 10000 rpm for 5 min. The absorbance was measured at 510 nm using ascorbic acid as positive control. A_0 was measured using distilled water (instead of sample solution) as blank, and A_{x0} was measured using distilled water (instead of H_2O_2) as control. The hydroxyl radical scavenging activity was expressed as follows:

$$\text{Scavevging rate } (\%) = \frac{A_0 - (A_x - A_{x0})}{A_0} \times 100\%$$

2.6.3. Detection of DNA cleavage

Protection against DNA damage was evaluated as described previously (Akhilesh & Sharmila, 2007; Russo, Acquaviva, & Campisi, 2000). Three microliters of pUC-19 plasmid DNA (10 nM), 4 µL of 2% H_2O_2, and 3 µL of 5 mg/mL sample were added to a microfuge tube. The reaction was initiated by UV irradiation for 15 min on a clean bench at room temperature. Reactionmixtures were subjected to 1% agarose gel electrophoresis to determine the extent of DNA damage.

2.6.4. Protective effect of polysaccharides on H_2O_2-induced toxicity in SH-SY5Y cells

Cytoprotective activity of polysaccharides on H_2O_2-induced cell injury was assessed by the MTT assay (Nirmaladevi et al., 2014). The human neuroblastoma SH-SY5Y cells (2×10^4/mL) were plated in sterile 96-well plates and incubated for 24 h to allow cells to adhere to the plate. Cells pretreated for 2 h with various concentrations of polysaccharides (5, 10, 25, 50, 100 µg/mL) were treated with H_2O_2 (400 µmol/L) for 24 h at 37 °C in a humidified 5% CO_2 incubator. Then, each well was added with MTT (5 mg/mL) and incubated for another 4 h. After aspirating the

supernatant from the wells, 100 μL of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader. Each experiment was performed in triplicate.

2.7 Lymphocyte proliferation assay

Lymphocyte proliferation assays were assessed in vitro by an MTT-based colorimetric assay. C57 mice (8-12 weeks old) were sacrificed by cervical dislocation. Spleens were aseptically removed and placed in sterile RPMI-1640 medium, and single cell suspension was prepared by disrupting spleen into small pieces under aseptic conditions. The cells were filtered with gauze and red cells were lysed by adding 0.75% NH_4Cl. Cells were washed twice with RPMI-1640 and resuspended in RPMI-1640 media. Aliquots of 100 μL of splenocytes (5×10^6/mL) were placed in a 96-well culture plate. Samples (concentrations of 10, 20, 40, 80, 200 μg/mL) were added to each cell with or without ConA (5 μg/mL) or LPS (10 μg/mL) for 48 h at 37 °C in a humidified 5% CO_2 incubator. Then, each well was added with 20 μL of MTT (5 mg/mL) and the plate was incubated for another 4 h. After aspirating the supernatant from the wells, 100 μL of DMSO was added to dissolve the formazan crystals. Absorbance of each well was measured at 570 nm using a microplate reader. Control experiments were performed without the polysaccharide.

2.8 Statistical analysis

Data were presented as mean ± SD (standard deviation). Statistical analyses were performed by t-test and one way analysis of variance (SPSS, version 13.0). Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Isolation and purification of polysaccharide

DBFM was first separated through an anion-exchange DEAE-32 cellulose column and was eluted consecutively with distilled water and a linear gradient of NaCl from 0 M to 1.0 M. Three fractions corresponding to DBFN, DBFA1, and DBFA2 were clearly separated in the elution profile of the anion-exchange chromatogram (Fig. 1a). DBFN was a neutral polysaccharide, whereas both DBFA1 and DBFA2 were acidic polysaccharides. Each of the three fractions was further purified by Sepharose CL-6B column chromatography using 0.9% NaCl as eluent. A fraction from DBFA2 showing a single and symmetrically sharp peak was collected and designated as DBFM3 (Fig. 1b).

3.2. Molecular weight and homogeneity of polysaccharides

The homogeneity and molecular weight of DBFM3 was determined by HPGLC. The profile of DBFM3 showed a single and symmetrical narrow peak (Fig. 2), which indicated that DBFM3 was a homogeneous and highly pure polysaccharide. HPGLC analysis revealed that the molecular weight of DBFM3 was 1.8×10^5 Da, making it a high molecular weight polysaccharide. A similar molecular weight has been reported for an immunostimulatory polysaccharide (2.1×10^5 Da) from *C. militaris* mycelium (Lee et al., 2010a, 2010b) and an acidic proteoglycan (1.5×10^5 Da) from *P. Linteus* (Kim, Park, & Nam, 2003). Some studies have confirmed that polysaccharides with

higher molecular weights exhibit higher antitumor and immunological activity. A high molecular weight is essential for enhancing the antitumor and immunological activities of polysaccharides (Yan, Pei, & Ma, 2015).

3.3. Chemical components and monosaccharide composition

As a homogeneous polysaccharide, DBFM3 was chosen for further study. Chemical analysis showed that the total sugar content, uronic acid, and protein content of DBFM3 were 57.64%, 20.29%, and 1.75% respectively.

The monosaccharide composition of DBFM3 was measured by HPLC analysis. Nine standard monosaccharides were separated rapidly within 30 min. The peaks of standard monosaccharides were identified in the order of D-mannose, D-glucuronic acid, L-rhamnose, galacturonic acid, D-glucose, D-galactose, D-xylose, L-arabinose, and L-fucose (Fig. 3a). The sample was identified by matching retention time with those of standard monosaccharides under the same analytical conditions. DBFM3 was composed of mannose, galacturonic acid, and galactose at a molar ratio of 1:18.16:0.702. Galacturonic acid accounted for the largest proportion, indicating that DBFM3 was a pectin-type polysaccharide just like the pectin polysaccharide PM II_{50} reported by Samuelsen et al. (1995)

3.4. Structural characterization of DBFM3

The UV absorption spectra of DBFM3 suggested that the polysaccharide had a low absorption at the wavelength of 280 nm, indicating that DBFM3 contained trace protein.

FT-IR spectroscopy is commonly used to determine organic the functional groups (Wu, Zhu, & Zhang, 2012) in molecules. As shown in Figure 4, the spectrum of DBFM3 displayed a broad and intense peak at 3366.64 cm^{-1} (3500-3100 cm^{-1}), which could be ascribed to the hydroxyl groups stretching vibration of polysaccharide and water involved in hydrogen bonding. The weak absorption peak in the 2930.33cm^{-1} region (3000-2800 cm^{-1}) was the characteristic of C-H stretching vibration of the methyl group (Seedevi, Moovendhan, Viramani, & Shanmugam, 2017) and the strong absorption at 1651.70 cm^{-1} was due to the C=O stretching vibration. The band at 1400.57 cm^{-1} represented the C-H bending vibration. Absorption peaks were detected in the 1700-1300 cm^{-1} region, which indicated that the polysaccharide had a carboxyl group (i.e., glycuronic acid in DBFM3). The absorbance at 1239.36 cm^{-1} was attributed to the existence of sulfate radical. The stretching peak at 1086.72 cm^{-1} indicated the presence of pyranose ring (Park, 1971). The band at 819 cm^{-1} was attributed to the D-galactopyranose unit (Barker, Bourne, & Stacey, 1954).

Signals corresponding to polysaccharide in the NMR spectra were identified on the results of monosaccharide analysis and chemical shifts (Hu et al., 2011; Yang, Lv, & Tian, 2010). The ^{1}H-NMR spectrum of DBFM3 was complicated due to the overlap of most signals. It was difficult to confirm the anomeric proton signals which were merged with the signal from HOD (Fig. 5a). However, ^{1}H-^{1}H COSY revealed three correlation peaks in the anomeric proton region (Fig. 5c), indicating the existence of three anomeric protons at δ 4.329, 4.599 and 4.642, which were assigned to H-1 of (1→6) -linked, (1→3,6) -linked and (1→3) -linked glucosyl residues,

respectively. The peaks of DBFM3 at around δ 4.7 were attributed to the chemical shifts of D_2O. Furthermore, δ 5.8-8.7 represented the aromatic protons, and weak signals at δ 0.6-3.2 indicated the ^1H chemical shift signal of β-H and γ-CH_3, as well as -CH_2 and -CH from protein amino acid residues, which indicated that DBFM3 was a glycopeptide.

The ^{13}C-NMR spectrum of DBFM3 (Fig. 5b) suggested that the resonances of 98-109 ppm were anomeric carbon (Hu et al., 2011)，further indicating a β-anomeric configuration for glucopyranosyl units (Hall & Johnson, 1969). The peak at 109.92 ppm was assigned to the C-1 signal of galactose. The signal at δ 61.009 should be unsubstituted C-6 of (1→3)-linked glucosyl residues. In addition, the ^{13}C-NMR spectrum showed a signal at low field from δ 160-180, which indicated the presence of uronic acid.

3.5. Antioxidant activity test analysis

3.5.1. DPPH scavenging assay

DPPH is a stable organic radical with peak absorption at 517 nm in methyl alcohol, which can be extensively used to evaluate the free-radical scavenging ability. As shown in Figure 6a, DBFM3 and DBFM demonstrated a dose-dependent DPPH scavenging ability ranging from 0.5 mg/mL to 5 mg/mL. The DPPH scavenging ability of DBFM3 was significantly stronger than that of unpurified DBFM with approximately 14.25 % at 5 mg/mL, but lower than that of the positive control.

3.5.2. Hydroxyl radical scavenging assay

Hydroxyl radical is one of the most reactive oxygen radicals and can induce severe damage to functioning biomolecules in living cells. Removing hydroxyl radicals is important for protecting living systems. As shown in Figure 6b, with increasing concentration, the scavenging capacity of DBFM3 and DBFM improved ranging from 0.5 mg/mL to 5 mg/mL. DBFM3 exhibited higher scavenging activity (with approximately 13.5 % at 5 mg/mL) on hydroxyl radical than unpurified DBFM, but lower than that of ascorbic acid.

3.5.3. Detection of DNA cleavage

The protective effect of DBFM3 on the damage caused by H_2O_2 and UV was evaluated using pUC-19 plasmid DNA. DNA damage was detected by gel electrophoresis. The results showed that DNA can be damaged by ·OH generated from UV irradiation (Fig. 7a). UV irradiation of samples containing rutin, which has strong UV absorption, revealed the presence of two forms of DNA, i.e., supercoiled (SC)-DNA and open circular (OC)-DNA (Lane 2, Fig. 7a), indicating that rutin can protect DNA. For this reason, we used rutin as positive control. Addition of DBFM3 revealed the existence of SC-DNA (Lane 3, Fig. 7a), indicating that DBFM3 had some protective effect against DNA damage. SC-DNA was completely converted into OC-DNA in the negative control to which PBS had been added instead of rutin (Lane 4, Fig. 7a), indicating that the selected conditions met the test requirements. Thus, polysaccharide had protective effect on DNA damage, which could be attributed to scavenging of hydroxyl radicals; this ability was associated with the antioxidant property of polysaccharides.

3.5.4. Protective effect of polysaccharide on H_2O_2-induced toxicity in SH-SY5Y cells

After exposure to H_2O_2 (400 μmol/L) for 24 h, cell viability decreased by 80 ± 2.08% compared to the normal group. However, pretreatment of cells with various concentrations of DBFM and DBFM3 significantly restored cell viability in a dose-dependent manner compared to the H_2O_2-model group (Fig. 7b). DBFM and DBFM3 at 50 μg/mL almost neutralized H_2O_2-induced oxidative stress demonstrating the effectiveness of polysaccharide in preventing oxidative stress to neuronal cells.

Some studies have indicated that the antioxidant activity of polysaccharides is related to many factors, including monosaccharide composition (Gao et al., 2013), uronic acid, and molecular weight. In addition, high GalA-containing polysaccharides could have high antioxidant activity, owing to the functional group-COOH. It has been shown that the functional groups (-COOH and CH_3CO-) are good electron or hydrogen donors and may thereby contribute to the antioxidant activity of polysaccharides (Wang et al., 2015). In our study, DBFM3 was found to be mainly composed of galacturonic acid, which contribute to the antioxidant activity of polysaccharides.

3.6 Lymphocyte proliferation assay

DBFM3 and DBFM were subjected to immune tests to evaluate their effects on lymphocyte proliferation. As shown in Figure 8a, splenic lymphocyte proliferation was stimulated by polysaccharide alone, and DBFM3 had a greater impact than

DBFM. As shown in Figure 8b and Figure 8c, synergistic effect was observed between polysaccharide and ConA or LPS. Lymphocyte proliferation was higher in the DBFM3-treated groups compared to the DBFM-treated group ($p<0.05$). Many polysaccharides have been demonstrated to possess immune modulation activity. For example, purified polysaccharides from mycelial cultures of *Phellinus linteus* have been found to stimulate the proliferation of T and B lymphocytes (Hanet al., 1999; Kim et al., 1996), which polysaccharides from *P. linteus* act as immunopotentiators partly by protecting T cells and enhancing mucosal IgA responses (Li et al., 2011). Previous studies have demonstrated that structural features such as β-(1→3) linkages and β-(1→6) linkages are important for increasing immune-competent cell activity (Wasser, 2002). The result of our study demonstrated that glycosidic linkage may be had related to the immune modulation activity of DBFM3.

(a) Polysaccharides, values represent mean ± SD; *P < 0.05, **P < 0.01 vs. negative control; (b) Polysaccharides + ConA, values represent mean ± SD; *P < 0.05, **P < 0.01 vs. ConA;

(c) Polysaccharides + LPS, values represent mean ± SD; *P < 0.05, **P < 0.01 vs. LPS.

4. **Conclusion**

A new polysaccharide, named DBFM3 in this paper, was isolated from *B. fumosa* mycelia. The water-soluble polysaccharide was purified by DEAE-32 cellulose and Sepharose CL-6B column chromatography. HPGLC showed that DBFM3 is

homogenous with a molecular weight of 1.8×10^5 Da. DBFM3 was found to be composed of mannose, galacturonic acid, and galactose at molar ratios of 1:18.16:0.702. Spectral analysis suggested that DBFM3 has (1→6), (1→3,6) and (1→3) linkages and pyranose conformation. Moreover, DBFM3 showed positive antioxidant potential on DPPH and hydroxyl radical invitro, as well as protective effect against DNA damage and SH-SY5Y cell damage induced by H_2O_2. Proliferation study on immunological activities indicated that DBFM3 has the potential to promote splenocyte proliferation, and increased the proliferation of lymphocytes in the presence of ConA or LPS as mitogen. These findings indicate that DBFM3 might be a candidate for the development of antioxidative or immunopotentiating agents in food and pharmaceutical industries.

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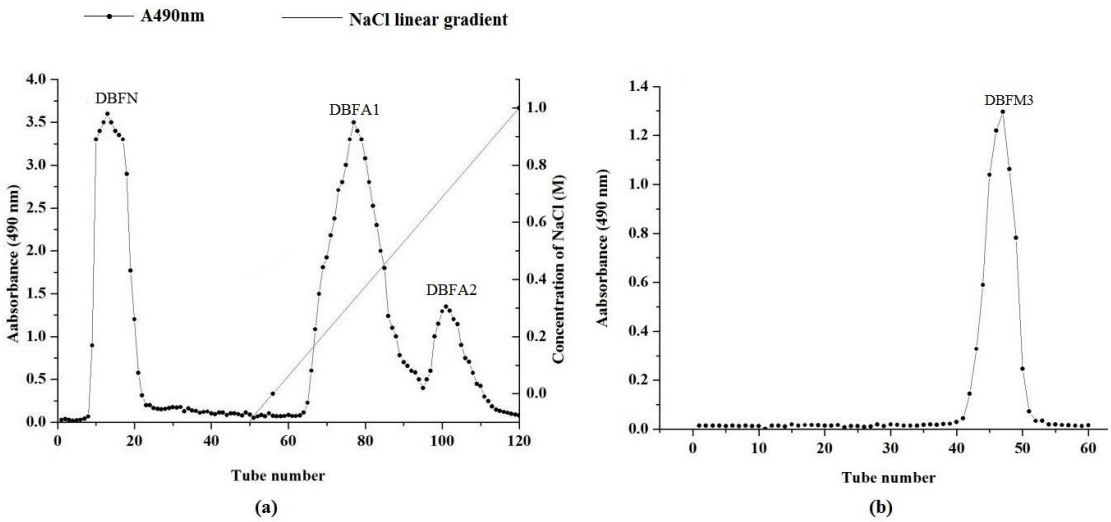

Fig. 1. Elution profile of DBFM on DEAE-32 cellulose column (a) and elution curve of polysaccharide fraction DBFA2 on Sepharose CL-6B column (b).

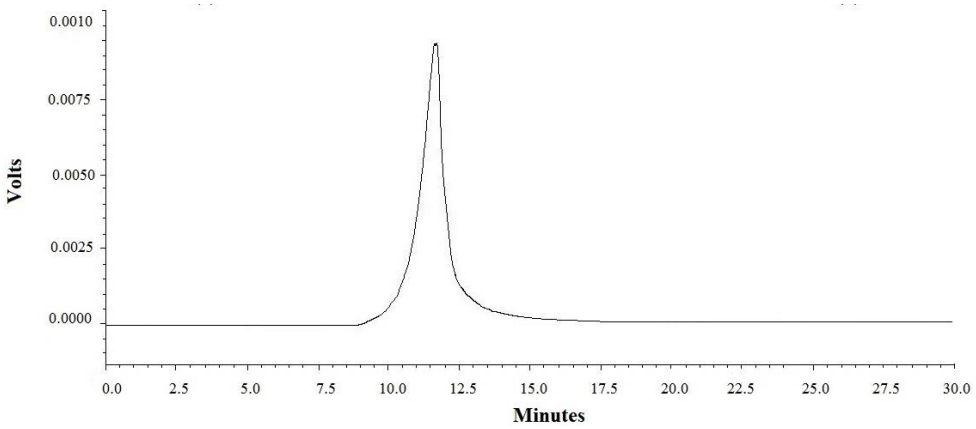

Fig. 2. HPGLC Profile of DBFM3

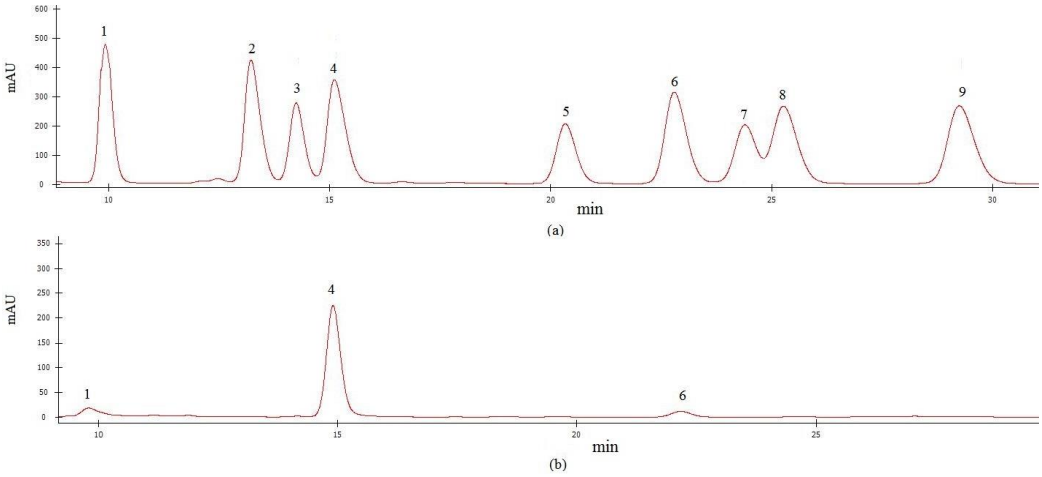

Fig. 3. HPLC spectra of derivatives of (a) standard monosaccharides and (b) DBFM3. Peaks: (1) D-mannose, (2) D-glucuronic acid, (3) L-rhamnose, (4) galacturonic acid, (5) D-glucose, (6) D-galactose, (7) D-xylose, (8) L-arabinose, and (9) L-fucose.

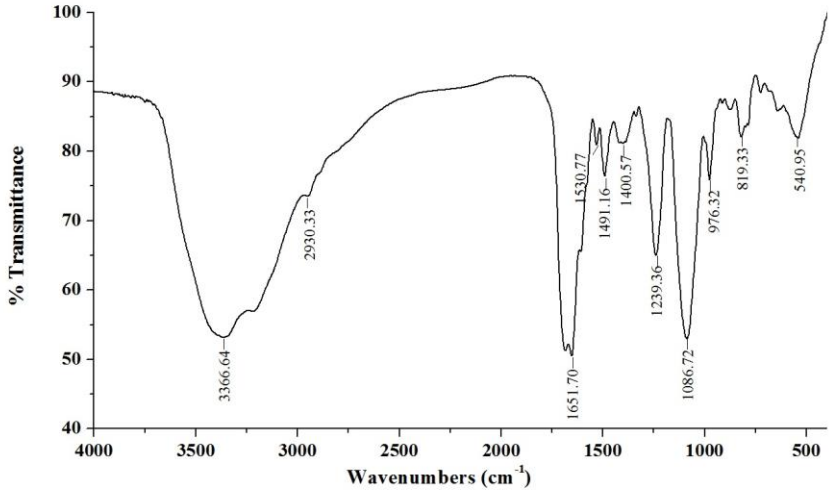

Fig. 4. FT–IR spectrum of DBFM3 from *B. fumosa*

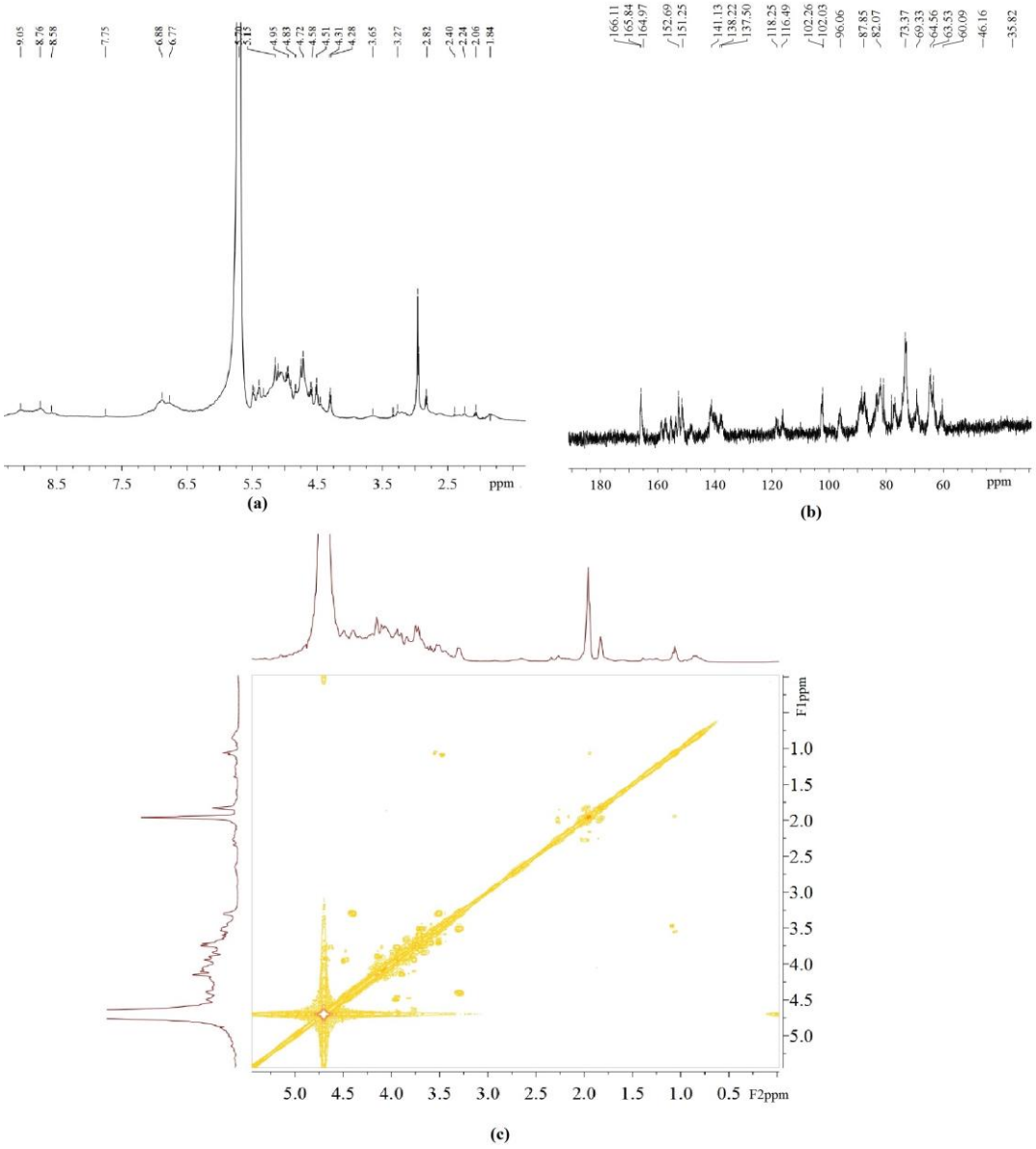

Fig. 5. NMR spectra of DBFM3 from *B. fumosa*: (a) ^{1}H-NMR, (b) ^{13}C-NMR, (c)^{1}H-^{1}H COSY spectrum.

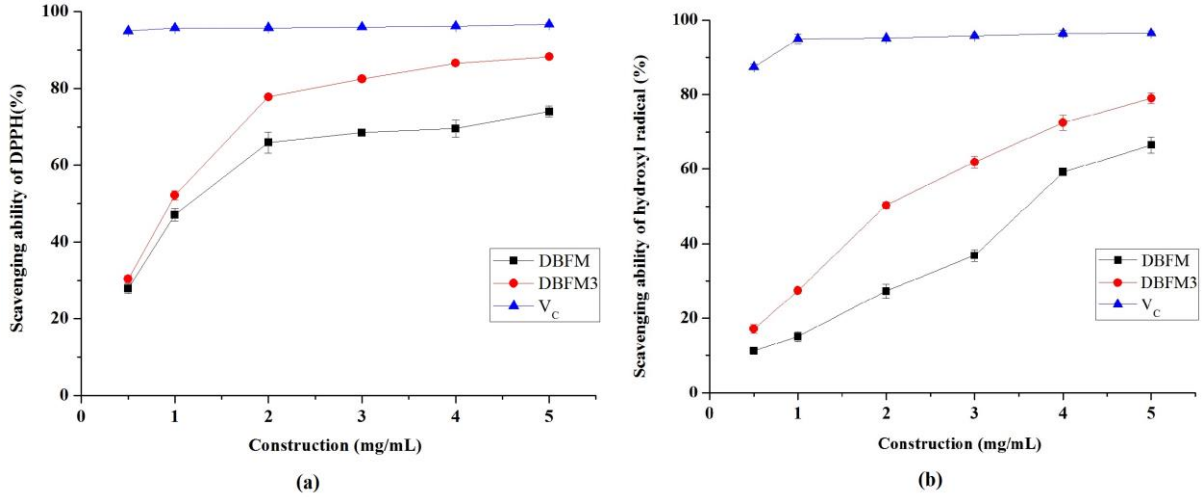

Fig. 6. Scavenging effects on DPPH (a) and hydroxyl radicals (b). DBFM: unpurified deproteinized polysaccharide; DBFM3: purified fraction of DBFM; V_C: positive control.

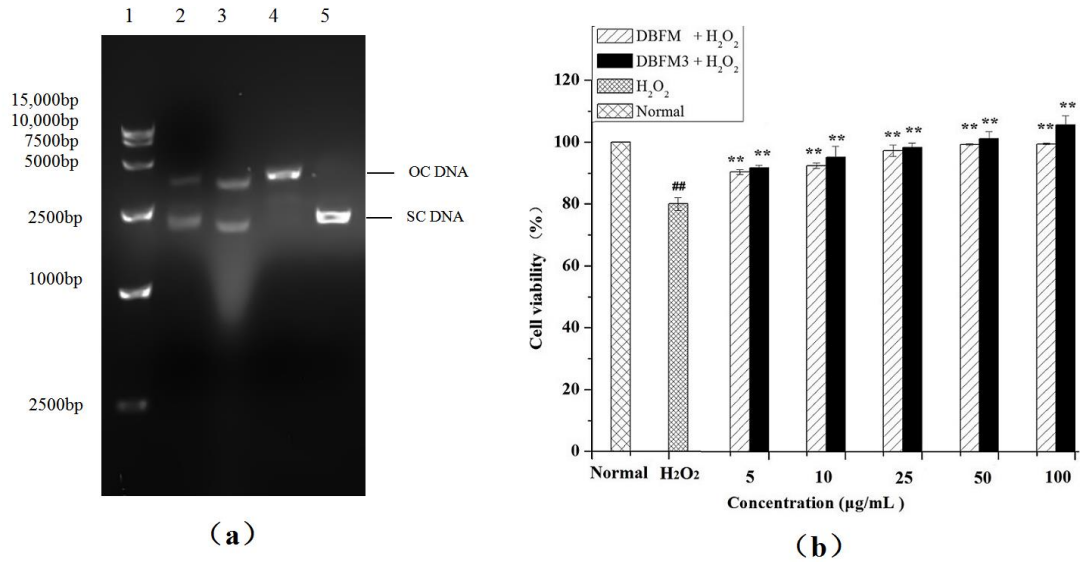

Fig. 7. (a) Protective effects of DBFM3 on pUC-19 DNA damage. DNA under different treatment conditions for 15 min; lanes 1 to 5 represent (1) marker, (2) rutin + H_2O_2 + DNA, (3) DBFM3 + H_2O_2 + DNA, (4) PBS + H_2O_2+ DNA, and (5) control (untreated DNA). OC and SC represent open circular and super coiled DNA, respectively. (b) Protective effect of DBFM and DBFM3 on viability of SH-SY5Y cells injured by H_2O_2. Values are mean ± SD, $^{\#\#}$ $P < 0.01$ vs. the normal group. *$P < 0.05$ and **$P < 0.01$ vs. the H_2O_2-model group.

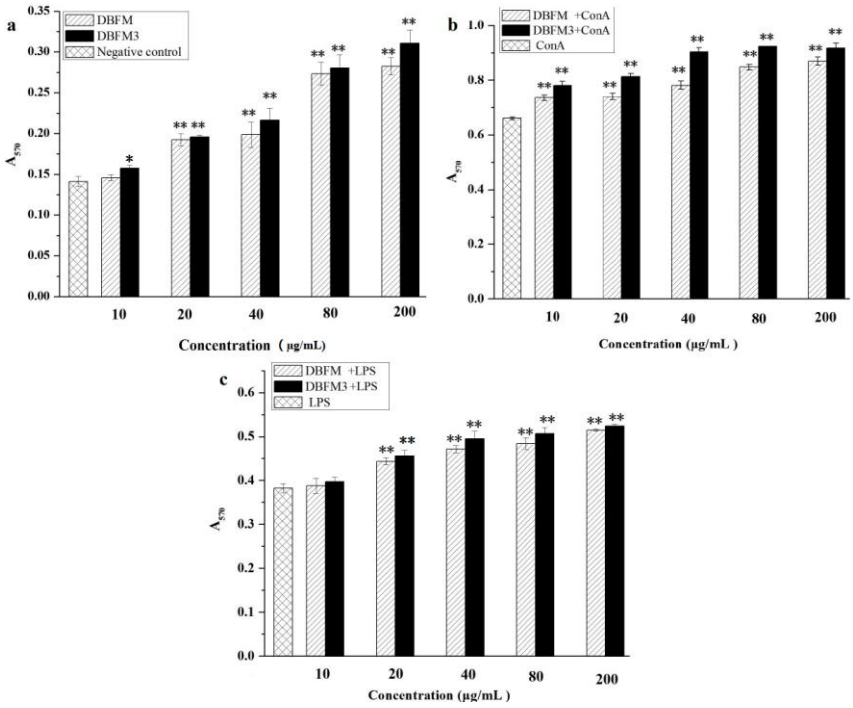

Fig. 8. Effect of DBFM3 and DBFM with or without ConA or LPS on splenocyte proliferation.