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Structural elucidation and antioxidant activity of a water-soluble polysaccharide from the fruit bodies of *Bulgaria inquinans* (Fries)

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ABSTRACT

The non-lichenized ascomycete *Bulgaria inquinans* (Fries), growing in the Changbai Mountain of China, has been used as medicinal diet for many years. In a previous study, we have reported that a heteropolysaccharide BIWS-4b from the fruit bodies of *B. inquinans* (Fries) exhibited markedly antimalarial and immunostimulating activities. In this paper, the structural features and antioxidant activity of BIWS-4b were investigated. The results showed that BIWS-4b contains an α-(1 → 2), (1 → 6)-mannan core to which the glucogalactan chains are attached. The glucogalactan chains were composed of (1 → 6)-, (1 → 5)- and (1 → 5,6)-linked β-Gal*f*, (1 → 4)-linked and non-reducing terminal β-Glc*p* units, and might be attached to the mannan core at the *O*-2 positions of α-Man*p* units. The antioxidant assays showed that BIWS-4b exhibited good activities, including free radicals scavenging effects, ferrous ion-chelating ability and reducing power. Thus, BIWS-4b could be used as a natural antioxidant agent for food and pharmaceutical industries.

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1. Introduction

Oxidative stress imposed by reactive oxygen species (ROS) may directly or indirectly cause tissue damage; induce many human diseases including cancers; and promote atherosclerosis, inflammation and aging. Antioxidants can disrupt radical chain reactions by interrupting iterations of free-radical-induced oxidation and hydrogen-donation. Thereby, stable free-radicals are formed that cannot initiate or propagate further lipid oxidation (Wade, Jackson, Highton, & Van Rij, 1987). However, the side effects of synthetic antioxidants may be responsible for some observed liver damage and carcinogenesis (Soubra, Sarkis, Hilan, & Verger, 2007). Thus, it is essential to find new antioxidant agents from natural sources that can protect the human body from free radicals without side effects.

Recently, it has been found that polysaccharides have many potent biological and pharmacological activities, including immunostimulation as well as anti-tumor, anti-virus, anti-inflammatory and hypoglycemic activities (Richards & Lowary, 2009; Wang et al., 2010). Mushroom polysaccharides have recently become attractive as food and as sources for the development of drugs. Several polysaccharides from fungi have exhibited strong antioxidant effects that are relevant to their health-protecting functions (Tsai, Song, Shih, & Yen, 2007; Tseng, Yang, & Mau, 2008). Therefore, polysaccharides from fungi have attracted considerable interest from for the food and pharmaceutical industries in developing nontoxic natural antioxidants that demonstrate measurable health benefits.

The non-lichenized, wood-inhabiting ascomycete *Bulgaria inquinans* (Fries) naturally grows in the Changbai Mountain area of China and has been used as food and folk medicine for many years. Small molecular compounds, such as benzofluoranthrene derivatives, dihydroxyperylenequinones and azaphiones from the fruit bodies of *B. inquinans* (Fries) have been isolated and tested for antitumor (Yang et al., 1993), antipruritic and antierythema activities (Jiang, Tsumuro, Takubo, Fujii, & Kamei, 2005). In our previous study, we isolated a heteropolysaccharide (BIWS-4b) composed of mannose, glucose and galactose from the fruit bodies of *B. inquinans* (Fries) (Bi et al., 2011). The initial structural features of BIWS-4b were established and its activity against malaria was evaluated. In this paper, we bring more information about the structural features of BIWS-4b and evaluated its antioxidant activities *in vitro*.

2. Materials and methods

2.1. Materials

Fruit bodies of *B. inquinans* (Fries) were collected from Changbai Mountain, which is in Fusong County within the Jilin Province of China. A voucher specimen (No. 20070802) was deposited at the

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School of Life Sciences, Northeast Normal University. 1,2,3-phentriol, potassium ferricyanide [$K_3Fe(CN)_6$], horseradish peroxidase (HRPase, 300 U/mg), 2-thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), nitro blue tetrazolium salt (NBT), ascorbic acid, ferrozine [3-(2-pyridy)-5,6-bis-(4-phenylsulfonicacid)-1,2,4-triazine, monosodium salt], phenazine methosulfate (PMS), dihydronicotineamidadenine dinucleotide (NADH), thiobarbituric acid (TBA) and deoxyribose, ethylenediamine tetraacetic acid (EDTA) were purchased from Sigma Co. (St. Louis, USA). Sepharose CL-6B and Sephadex G-75 were obtained from Pharmacia Co. (Sweden). All other reagents were of analytical grade and made in China.

2.2. Partial hydrolysis of BIWS-4b

BIWS-4b was isolated and purified from fruit bodies of *B. inquinans (Fries)* as described in our previous study (Bi et al., 2011). BIWS-4b (50 mg) was hydrolyzed with 5 mL of 0.15 M CF_3COOH for 5 h at 100 °C (Priteo, Leal, Bernabe, & Hawksworth, 2008). After dialysis the degraded polysaccharide (BIWS-4b-D) was then recovered by lyophilization.

2.3. Monosaccharide composition analysis

The monosaccharide analysis was conducted as described by Honda et al. (1989) and reported in our previous study (Bi et al., 2011).

2.4. Periodate oxidation

The periodate oxidation analysis was performed as described by Chaplin and Kennedy (1994). The sample (25 mg) was dissolved in 0.015 M $NaIO_4$ (25 mL), and the solution was kept at 4 °C in dark. The A_{223nm} of the reaction solution was measured every 6 h with a spectrophotometer. After the oxidation reaction was complete (48 h), the excess $NaIO_4$ was decomposed with ethylene glycol (0.1 mL). The amount of $NaIO_4$ consumption was calculated according to the decrease in absorbance at 223 nm. Formic acid production was determined by titration with 0.1 M NaOH. The reaction mixture was dialyzed against tap water and then distilled water, and then, it was later reduced by $NaBH_4$ overnight. After neutralization and dialysis, the retentate was freeze-dried, hydrolyzed with 2 M CF_3COOH (1 mL) at 120 °C for 2 h, reduced by $NaBH_4$, acetylated with pyridine (0.5 mL) and acetic anhydride (0.5 mL) at 90 °C for 1 h and then analyzed for sugar composition by gas chromatography (GC). GC was performed on a Shimadzu GC-14C instrument equipped with a hydrogen flame ionization detector and an Rtx-2330 column (0.32 mm × 15 m i.d., 0.2 μm).

2.5. Methylation analysis

The methylation analysis was carried out according to the method of Needs and Selvendran (1993) as reported in our previous study (Bi et al., 2011).

2.6. NMR analysis

The ^{13}C NMR spectrum was recorded using a Bruker 5-mm broadband observe probe at 20 °C with a Bruker Avance 600 MHz spectrometer (Germany), operating at 150 MHz. Sample (20 mg) was dissolved in D_2O (99.8%, 0.5 mL), freeze-dried, redissolved in D_2O (0.5 mL) and centrifuged to remove any excess sample. The experiment was recorded using standard Bruker software.

2.7. Determination of antioxidant activity

2.7.1. DPPH radical-scavenging activity assay

The scavenging activity of the DPPH free radical was assayed as reported by Shimada, Fujikawa, Yahara, and Nakamura (1992), with slight modification. Briefly, 4 mL of sample solution (0.25–4 mg/mL) in distilled water was mixed with 1 mL of 0.1 mM methanolic DPPH solution. Distilled water was used as a control, and ascorbic acid for comparison. After vigorously shaken, the mixture stood for 15 min in dark and another 20 min at room temperature. Then the absorbance of mixture was measured at 517 nm. The DPPH radical scavenging activity (%) was calculated as follows: scavenging activity (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A is the absorbance.

2.7.2. Hydroxyl radical-scavenging activity assay

Hydroxyl radical-scavenging activity was determined as described by Halliwell, Gutteridge, and Aruoma (1987), with slight modification. In brief, 0.1 mL of sample solution (0.25–4 mg/mL) was mixed with 0.6 mL of reaction buffer (containing 35 mM pH 7.4 phosphate buffer, 10 mM deoxyribose and 170 mM EDTA), 0.1 mL of 10 mM H_2O_2, 0.1 mL of 2 mM ascorbic acid and 0.1 mL of 1 mM ferric chloride. Distilled water was used as control, and ascorbic acid for comparison. Following incubation at 37 °C for 90 min, the reaction mixture was added 1 mL of 1% (w/v) TCA and 1 mL of 1% (w/v) TBA, and then heated in a boiling-water bath for 15 min. Finally, the mixture was measured by absorbance at 532 nm, and the hydroxyl radical-scavenging activity (%) was calculated as follows: scavenging activity (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A is the absorbance.

2.7.3. Superoxide anion-scavenging activity assay

The superoxide radical scavenging activity was assayed in the NADH-NBT-PMS system (Liu, Ooi, & Chang, 1997). 1 mL of sample solution (0.25–4 mg/mL) was added 1 mL of 300 μM NBT, 1 mL of 936 μM NADH, and 1 mL of 120 μM PMS in 100 mM phosphate buffer (pH 7.4) in sequence. Distilled water was used as control, and ascorbic acid was used for comparison. After incubation at 25 °C for 5 min in the dark, the mixture was measured at A_{560nm}, and the superoxide radical-scavenging activity (%) was calculated by the following equation: scavenging activity (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A is the absorbance.

2.7.4. H_2O_2-scavenging activity assay

The H_2O_2 scavenging activity was assayed by the method of Pick and Mizel (1981). 1 mL of sample solution (0.25–4 mg/mL) was mixed with 400 μL of 5 mM H_2O_2 solution and incubated for 20 min at room temperature. Distilled water was used as control, and ascorbic acid was used for comparison. The mixture was supplemented with 600 μL of HRPase-phenol red solution (300 μg/mL HRPase and 4.5 mM phenol red in 100 mM phosphate buffer). 10 min later, the mixture was measured at A_{610nm}, and the hydrogen peroxide scavenging activity (%) was calculated by the following equation: scavenging activity (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A is the absorbance.

2.7.5. Chelating effect on ferrous ion assay

The chelating activity on ferrous ions was measured as described by Dinis, Madeira, and Almeida (1994). 1 mL of sample solution (0.25–4 mg/mL) was mixed with 0.1 mL of 2 mM $FeCl_2$ for 30 s, and then reacted with 0.2 mL of 5 mM ferrozine for 10 min at room temperature. Distilled water was used as control, and ascorbic acid was used for comparison. The mixture was measured at A_{562nm}, and the chelating activity (%) was calculated by the following equation: scavenging activity (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A is the absorbance.

2.7.6. Reducing power assay

The reducing power was determined referring to the ferric-reducing antioxidant power (FRAP) assay (Yuan, Carrington, & Walsh, 2005). 1 mL of sample solution (0.25–20 mg/mL) was mixed with 2.5 mL of 0.2 M pH 6.6 phosphate buffer and 2.5 mL of 1% (w/v) $K_3Fe(CN)_6$, then incubated at 50 °C for 20 min. Distilled water was used as control, and ascorbic acid was used for comparison. 1 mL of 10% (w/v) TCA was added to the mixture and centrifuged (3000*g* × 10 min). 2.5 mL of the upper-layer solution was mixed with 2.5 mL of distilled water and 2.5 mL of 0.1% (w/v) $FeCl_3$. The absorbance of mixture was measured at 700 nm, and the reducing power has a positive correlation with the A_{700nm}.

2.7.7. Self-oxidation of 1,2,3-phentriol assay

The scavenging activity for self-oxidation of 1,2,3-phentriol was evaluated according to the method of Marklund and Marklund (1974). Briefly, 0.1 mL of sample solution (0.25–10 mg/mL) was mixed with 2.8 mL of 50 mM pH 8.0 Tris–HCl buffer (containing 1 mM EDTA) and 0.2 mL of 6 mM 1,2,3-phentriol. Distilled water was used as control, and ascorbic acid was used for comparison. After rapidly shaken, the absorbance of mixture was measured at 325 nm per 30 s for 4 min against a blank, and a slope was calculated as absorbance of per min. The scavenging activity for self-oxidation of 1,2,3-phentriol was calculated by the following equation: scavenging activity (%) = (1 − slope of sample/slope of control) × 100.

2.8. Statistical analysis

All data were expressed as the mean ± standard deviation (SD) of six replicates, and subjected to the analysis of variance for a completely random experimental design; a one-way ANOVA analysis of variance and a Student's *t*-test were conducted to identify differences among means. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

3.1. Structural analysis of BIWS-4b

In our previous report, a homogeneous heteropolysaccharide BIWS-4b from *B. inquinans* (Fries) was purified and its anti-malarial activity was tested (Bi et al., 2011). BIWS-4b is consisted of mannose, glucose and galactose (1.0:0.57:2.1), molecular weight 7.4 kDa. Its initial structural features were analyzed by methylation, FT-IR and ^{13}C NMR. The Gal residues exist in the forms of (1 → 6)-, (1 → 5)-, and (1 → 5,6)-linked β-Gal*f*, Glc residues in the forms of (1 → 4)-linked and nonreducing terminal β-Glc*p*, and Man residues in the forms of (1 → 2)-, (1 → 6)-, (1 → 2,6)-linked and non-reducing terminal α-Man*p*.

In the present study, the structural features are further investigated by periodate oxidation and partial acid hydrolysis. The periodate oxidation showed that 1.23 mol was consumed and 0.25 mol of formic acid was produced per mole of glycosyl residues on average. GC analysis of the oxidized product indicated that the hydrolysates contained erythritol (43.7%) and glycerol (55.6%). The erythritol was from 6-*O*-substituted, 5-*O*-substituted and 5,6-di-*O*-substituted Gal*f* units in addition to 4-*O*-substituted Glc*p* units. The glycerol was from the non-reducing terminals of Glc*p* and Man*p* as well as from 2-*O*-substituted, 6-*O*-substituted and 2,6-*O*-substituted Man*p* units.

Subsequently, BIWS-4b was treated with diluted acid to selectively hydrolyze furanosidic chains, producing a degraded polysaccharide fraction (BIWS-4b-D, 19.4% yield). BIWS-4b-D was composed exclusively of mannose. Methylation analysis demonstrated that terminal Man*p* in addition to 2-*O*-substituted, 6-*O*-substituted and 2,6-*O*-substituted Man*p* residues were present in the degraded polysaccharide fraction (Table 1), which is consistent with ^{13}C NMR results. According to the literatures (Ahrazem et al., 2007; Gomez-Miranda et al., 2004; Priteo et al., 2008; Tischer, Gorin, De Souza, & Barreto-Bergter, 2002), the signals at δ 101.2, 99.6, 98.3 and 97.2 in the ^{13}C NMR spectrum of BIWS-4b-D could be assigned to C-1 of differently linked α-Man*p* residues (Fig. 1, Table 2). Based on the above results, BIWS-4b-D consisted of a main chain of α-(1 → 6) mannopyranose substituted at *O*-2 with variable amounts single Man*p* units or with small chains of α-(1 → 2) Man*p* residues (Fig. 2). The structure of BIWS-4b-D was similar to those reported for the mannan cores obtained by partial hydrolysis of several other fungal polysaccharides (Ahrazem et al., 2007; Gomez-Miranda et al., 2004; Priteo et al., 2008; Tischer et al., 2002). Both Glc*p* and Gal*f* units were hydrolyzed simultaneously, implying that they might comprise glucogalactan chains attached to mannan chains *via* Gal*f* units. After removing the Glc*p* and Gal*f* units by mild hydrolysis, the ratios of non-reducing terminal and 2-*O*-substituted Man*p* increased, whereas the ratio of 2,6-di-*O*-substituted Man*p* decreased (Table 2). Based on these results, the glucogalactan chains were probably linked to the terminal residues of the (1 → 2)-Man*p* side chains or the *O*-2 positions of (1 → 6)-Man*p* of main chains.

3.2. Antioxidant activity analysis

3.2.1. Scavenging activity to the DPPH radical

The free radical of DPPH is stable and widely used to evaluate the free radical scavenging ability of natural compounds. The DPPH radical-scavenging activity is conceivably due to a

Table 1
Methylation analysis result of BIWS-4b and BIWS-4b-D.

Components[a]	Molar ratio[b]		Retention time (min)	Linkage type
	BIWS-4b[c]	BIWS-4b-D		
2,3,6-Me_3-Gal	4.6	n.d	17.95	→ 5)-Gal*f*-(1 →
2,3-Me_2-Gal	3.1	n.d	17.88	→ 5,6)-Gal*f* -(1 →
2,3,5-Me_3-Gal	7.6	n.d	18.94	→ 6)-Gal*f*-(1 →
2,3,6-Me_3-Glc	2.2	n.d	18.32	→ 4)-Glc*p*-(1 →
2,3,4,6-Me_4-Glc	2.6	n.d	17.11	Glc*p*-(1 →
3,4,6-Me_3-Man	2.4	3.4	17.84	→ 2)-Man*p*-(1 →
2,3,4,6-Me_4-Man	1.0	1.7	17.06	Man*p*-(1 →
3,4-Me_2-Man	1.2	1.0	18.82	→ 2,6)-Man*p*-(1 →
2,3,4-Me_3-Man	2.9	5.7	18.72	→ 6)-Man*p*-(1 →

[a] 2,3,6-Me_3-Gal = 1,4,5-tri-acetyl-2,3,6-tri-O-methyl galactitol, etc.
[b] Relative molar ratio to 2,3,4,6-Me_4-Man, calculated from the peak areas.
[c] The data reported in our previous paper (Bi et al., 2011).

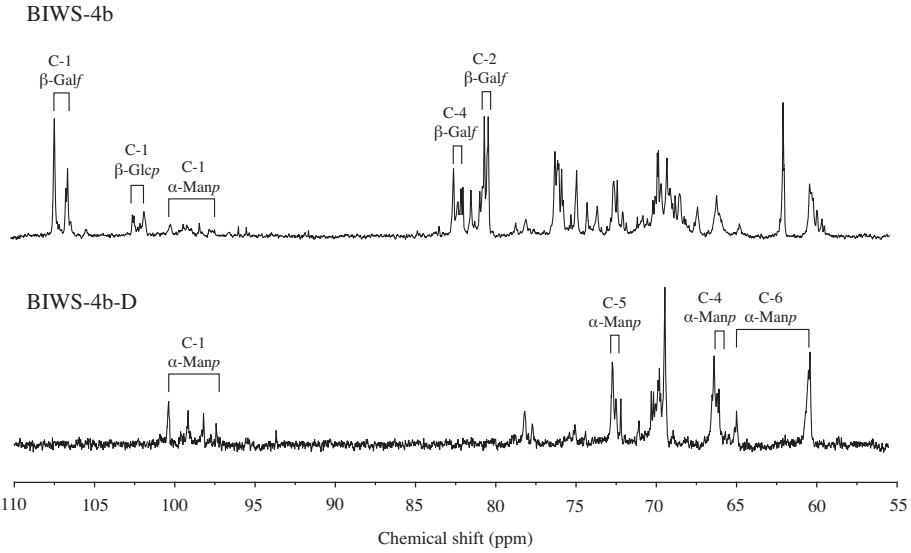

Fig. 1. ^{13}C NMR spectra of BIWS-4b and BIWS-4b-D.

Table 2
^{13}C NMR chemical shifts of BIWS-4b and BIWS-4b-D in D_2O.

Sample	Residue	δ ^{13}C (ppm)					
		1	2	3	4	5	6
BIWS-4b[a]	→ 5)-β-Galf-(1 →	107.7	80.9	76.8	84.0	75.5	62.7
	→ 5,6)-β-Galf-(1 →	107.0	81.2	76.4	83.1	74.2	68.0
	→ 6)-β-Galf-(1 →	106.9	81.5	76.7	82.8	69.9	69.7
	→ 4)-β-Glcp-(1 →	102.4	73.2	74.3	79.1	74.8	60.6
	β-Glcp-(1 →	102.2	73.0	74.2	69.1	75.8	60.3
	α-Manp-(1 →	101.2	69.1	69.6	66.5	72.4	60.1[b]
	→ 2)-α-Manp-(1 →	99.5	77.3[b]	68.8	66.7[b]	72.4	60.2[b]
	→ 6)-α-Manp-(1 →	98.4	69.1	69.6	66.6[b]	71.9	64.9[b]
	→ 2,6)-α-Manp-(1 →	97.3	77.6[b]	69.4	66.7	71.9	64.9[b]
BIWS-4b-D	α-Manp-(1 →	101.2	68.9	69.6	66.7	72.1	59.9
	→ 2)-α-Manp-(1 →	99.6	77.2	68.9	66.7	72.2	59.9
	→ 6)-α-Manp-(1 →	98.3	68.9	69.6	66.5	71.8	64.8
	→ 2,6)-α-Manp-(1 →	97.2	77.7	69.5	66.7	71.8	64.8

Underline bold numbers represent glycosylation sites.
[a] The data reported in our previous paper (Bi et al., 2011).
[b] These values may have to be interchanged.

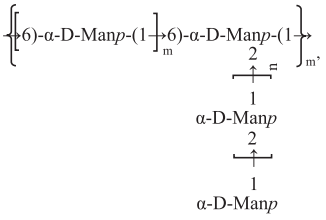

Fig. 2. Structure of BIWS-4b-D.

hydrogen-donating ability. As shown in Fig. 3a, BIWS-4b exhibited a dose-dependent scavenging activity at the tested concentrations. Its EC_{50} value was 0.74 mg/mL indicating that BIWS-4b had an appreciable DPPH radical scavenging activity. However, the inhibiting ability was lower than that ascorbic acid.

3.2.2. Scavenging activity to the hydroxyl radical

Hydroxyl radicals can be generated by reactions of iron–EDTA complexed with H_2O_2 in the presence of ascorbic acid; they tint the reaction pink when reacting with deoxyribose with heat and TBA under acidic conditions. The degree of pink tint was used to determine the amount of hydroxyl radicals present. As shown in Fig. 3a, the scavenging effect of BIWS-4b on hydroxyl radical was dose-dependent. The EC_{50} value of BIWS-4b was 0.55 mg/mL, and lower than that of ascorbic acid (0.65 mg/mL), suggesting a good hydroxyl radical scavenger effect.

3.2.3. Scavenging activity to the superoxide anion

The superoxide anion, one of the precursors to singlet oxygen molecules and hydroxyl radicals, indirectly initiates lipid peroxidation, so its scavenging is important to anti-oxidation. Superoxide radicals were generated in a PMS/NADH system and assayed by the reduction of NBT in this study. As shown in Fig. 3a, BIWS-4b exhibited a dose-dependent superoxide anion-scavenging effect, and its EC_{50} value was 0.95 mg/mL. Although the scavenging activity was lower than that of ascorbic acid, BIWS-4b did nevertheless show good scavenging activity toward superoxide anion radicals.

3.2.4. Scavenging activity to H_2O_2

H_2O_2 is not a free radical, but it plays role in radical formation as an intermediate in the production of ROS molecules. As shown in

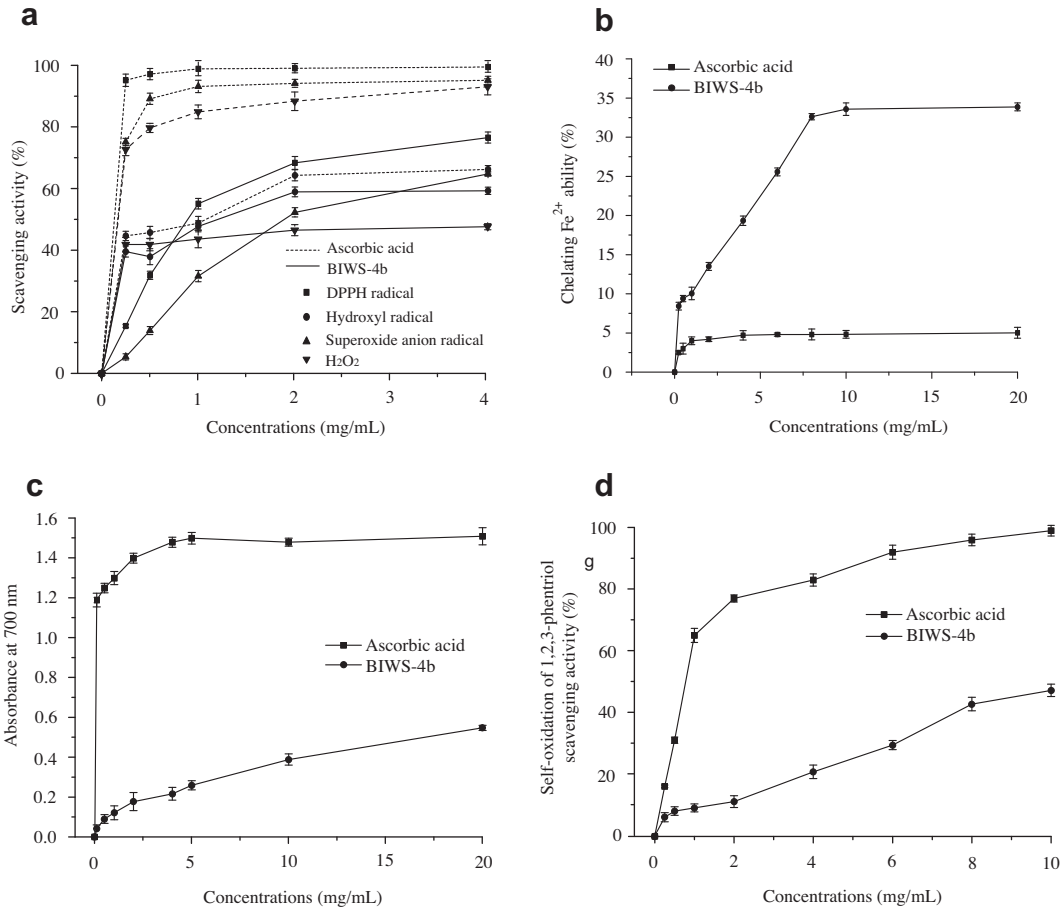

Fig. 3. Antioxidant activity of BIWS-4b from the fruit bodies of *Bulgaria inquinans* (Fries): (a) scavenging activities to DPPH-radical, hydroxyl radical, superoxide anion and H_2O_2, (b) chelating activity on ferrous ion, (c) reducing power, and (d) scavenging activity to self-oxidation of 1,2,3-phentriol; data are presented as the mean values ($n = 6$).

Fig. 3a, the H_2O_2 scavenging activity of BIWS-4b was 42.05–47.78% at the tested concentration (0.25–4 mg/mL), and reached plateau phase at a dose of 2 mg/mL. Although the H_2O_2-scavenging activity of BIWS-4b was lower than that of ascorbic acid in this study, the result suggested BIWS-4b had some scavenging effect on H_2O_2.

3.2.5. Chelating activity on the ferrous ion

The ferrous ion is an extremely reactive metal that will catalyze oxidative changes to lipids, proteins and other cellular components. Such oxidative damage is induced by hydroxyl radicals generated by the Fenton reaction. As shown in Fig. 3b, chelating ability of BIWS-4b exhibited a concentration-dependent manner. Its chelating ability was higher than that of ascorbic acid. Its chelating activity was 33.57% and reached plateau phase at a dose of 10 mg/mL. The result indicated that BIWS-4b had some chelating effect on ferrous ions.

3.2.6. Reducing power assay

The reducing power assay treats the antioxidants as reductants in a redox-linked colorimetric reaction. The resulting value reflects the reducing power of the antioxidant and may serve as a valuable indicator of potential antioxidant activity. As shown in Fig. 3c, the reducing power of BIWS-4b was dose-dependent. When the concentration increased from 0.25 mg/mL to 20 mg/mL, the A_{700nm} value for BIWS-4b increased from 0.046 to 0.549, suggesting some reducing power effect.

3.2.7. Scavenging activity to self-oxidation of 1,2,3-phentriol

1,2,3-phentriol rapidly autoxidizes in alkaline solution, and several intermediate products are apparently formed, such as $O_2^{\cdot-}$, which results in the solution becoming yellow–brown. The resultant absorbance spectrum shows a shoulder between 400 and 425 nm. Antioxidants can interfere with 1,2,3-phentriol autoxidation by scavenging $O_2^{\cdot-}$. As shown in Fig. 3d, scavenging activity of BIWS-4b on scavenging self-oxidation of 1,2,3-phentriol correlated well with increasing concentrations, and reached 47.18% at a dose of 10 mg/mL. This result indicates that BIWS-4b had weaker scavenging ability for the self-oxidation of 1,2,3-phentriol than that of ascorbic acid.

Recent research has shown that some polysaccharides rich in mannose, glucose or galactose play important roles as free radical scavengers, ferrous metal ion chelators and reducers for the prevention of oxidative damage in living organisms (Chen et al., 2011; Tian, Zhao, Guo, & Yang, 2011). For example, the polysaccharide from *Ganoderma lucidum*, containing (1 → 2)-, (1 → 3)-, (1 → 4)- and (1 → 6)-linked β-Glc*p* residues, exhibited free radical-scavenging and ferrous ion chelating abilities (Liu, Wang, Pang, Yao, & Gao, 2010). The polysaccharide from *Russula virescens*, containing (1 → 6),(1 → 2,6)-linked α-Gal*p* and non-reducing terminal α-Man*p*, had strong reducing power, free radical scavenging activity and Fe^{2+} chelating activity (Sun, Liu, Yang, & Kennedy, 2010). Some polysaccharides containing mannose residues had strong scavenging ability, such as the polysaccharide from *Dendrobium denneanum* containing (1 → 4),(1 → 6)-linked α-Glc*p* and non-reducing terminal α-Man*p* (Luo et al., 2011) as well as the

polysaccharide from *Edwardsiella tarda* containing (1 → 3)-linked α-Man*p* and (1 → 2)-linked β-Man*p* (Guo et al., 2011).

The polysaccharides containing β-galactofuranose residues and an α-mannan core from lichenized ascomycota and pathogens have been reported with many biological activities, such as anticoagulant, antithrombotic, immunomodulating and antitumor activities (Martinichen-Herrero, Carbonero, Sassaki, Gorin, & Iacomini, 2005; Wasser, 2002). However, there are no reports concerning the antioxidant activities of these polysaccharides. Here, we report the antioxidant activities of a polysaccharide consisting of β-galactofuranose and β-glucopyranose residues with an α-mannan core isolated from the non-lichenized edible mushroom *B. Inquinans* (Fries). Compared with other polysaccharides reportedly containing mannose, galactose and glucose, BIWS-4b had remarkable hydroxyl radical-scavenging and some ferrous ion-chelating abilities, which were even higher than those of ascorbic acid, a standard positive control. The mechanisms of antioxidant activity are complex, and polysaccharides may contribute to antioxidant activity in several paths. Therefore, seven different assays were employed to evaluate the potential antioxidant capacity of BIWS-4b. All of the assays displayed a similar trend in antioxidant activity. In summary, BIWS-4b has antioxidant activity via multi-mechanisms and it should be regarded as potential natural antioxidant.

4. Conclusion

BIWS-4b was identified to have a mannan core and glucogalactan side chains. The mannan core contained a main chain composed of α-(1 → 6) Man*p* residues and side chains composed of single Man*p* units or several α-(1 → 2) Man*p* residues. The glucogalactan chains were composed of (1 → 6)-, (1 → 5)- and (1 → 5,6)-linked β-Gal*f* and (1 → 4)-linked and non-reducing terminal β-Glc*p*, and attached to the *O*-2 positions of Man*p* residues of the mannan core. BIWS-4b had appreciable antioxidant activity *in vitro via* multi-mechanisms, which was evident by evaluating its scavenging activity toward free radicals and H_2O_2 and its ferrous ion-chelating ability and reducing power. BIWS-4b could be a potential natural antioxidant.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.11.039.

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