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Structural features and antitumor activity of a novel polysaccharide from alkaline extract of *Phellinus linteus* mycelia

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ABSTRACT

A novel high molecular weight polysaccharide (PL-N1) was isolated from alkaline extract of the cultured *Phellinus linteus* mycelia. The weight average molecular weight (M_w) of PL-N1 was estimated at 343,000 kDa. PL-N1 comprised arabinose, xylose, glucose, and galactose in the molar ratio of 4.0:6.7:1.3:1.0. The chemical structure of PL-N1 was investigated by FTIR and NMR spectroscopies and methylation analysis. The results showed that the backbone of PL-N1 comprised (1 → 4)-linked β-D-xylopyranosyl residues, (1 → 2)-linked α-D-xylopyranosyl residues, (1 → 4)-linked α-D-glucopyranosyl residues, (1 → 5)-linked β-D-arabinofuranosyl residues, (1 → 4)-linked β-D-xylopyranosyl residues which branched at O-2, and (1 → 4)-linked β-D-galactopyranosyl residues which branched at O-6. The branches consisted of (1 →)-linked α-D-arabinofuranosyl residues. Antitumor activity assay *in vitro* showed that PL-N1 could inhibit the growth of HepG2 cells to a certain extent in a dose-dependent manner. Thus, PL-N1 may be developed as a potential, natural antitumor agent and functional food.

1. Introduction

Edible and medicinal fungi are extensively applied to functional food and nutraceutical products because of their proven nutritive and medicinal properties (Ren, Reynisson, Perera, & Hemar, 2013; Stachowiak & Reguła, 2012). Polysaccharides, which are important biological response modifiers, represent a major class of bioactive molecules isolated from edible and medicinal fungi. These biomacromolecules have notable antitumor, immunomodulatory, and other medicinal properties (Giavasis, 2014; Ooi & Liu, 2000; Ramberg, Nelson, & Sinnott, 2010). To date, some fungal polysaccharides, such as lentinan from *Lentinus edodes*, schizophyllan from *Schizophyllum commune*, and Krestin from *Coriolus versicolor*, are marketed as anticancer or immunomodulation pharmaceutical agents in Japan and Korea (Zhang, Cui, Cheung, & Wang, 2007; Zong, Cao, & Wang, 2012). These polysaccharides and their derivatives have been proven useful against various cancers (Wasser, 2002).

Phellinus linteus (Berkeley & M. A. Curtis) Teng or Sanghuang is a medicinal fungus used as a prestigious tonic and therapeutic herb in Japan, China, and Korea (Zhu, Kim, & Chen, 2008). *P. linteus* has attracted much attention and interest in recent years because of its health-stimulating properties and medicinal applications (Hsieh, Wu, & Wu, 2013; Silva, 2010). This fungus exhibits a broad spectrum of biological activities, which includes immunomodulatory, antitumor, antioxidant, and antidiabetic properties (Kim et al., 2010; Kozarski et al., 2011; Li et al., 2011; Wu, Liaw, Pan, Yang, & Ng, 2013). Recent medicinal investigations have demonstrated that polysaccharides and polysaccharide-protein complexes are the most potential bioactive constituents of *P. linteus.*

The multiple biological activities of polysaccharides isolated from *P. linteus* are attributed to their diverse monosaccharide constituents and chemical structures. The precise chemical structures of *P. linteus* polysaccharides play an important role in understanding the relationship between structure and functional properties for future applications in nutrition and therapeutics. Some studies have successfully characterized the structures of polysaccharides isolated from different *Phellinus* species using hot water extraction. These species include *P. linteus* (Kim et al., 2010), *Phellinus igniarius* (Chen et al., 2011), *Phellinus baumii* Pilát (Ge, Mao, Zhang, Wang, & Sun, 2013), and *Phellinus ribis* (Liu & Wang, 2007). Limited reports on the structural features and biological activities of *Phellinus* polysaccharides obtained by other extraction media are available. We recently obtained three water-soluble polysaccharides from cultured *P. linteus* mycelia using hot water, 1% $(NH_4)_2C_2O_4$, and 1.25 M NaOH/0.05% $NaBH_4$ extraction method (Wang, Pei, Ma, Cai & Yan, 2014). The results demonstrated that water-soluble polysaccharides (PL-N) extracted by 1.25 M NaOH/0.05% $NaBH_4$ exhibited

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stronger biological activities compared with the others. However, the fine chemical structures of PL-N have not been reported yet.

Therefore, in the present study, the detailed structural characterization of a purified polysaccharide (PL-N1) from alkaline extract of the *P. linteus* mycelia was elucidated by combined chemical analysis and instrumental determinations, which included acid hydrolysis, methylation analysis, gas chromatography (GC), GC-mass spectrometry (MS), high-pressure gel permeation chromatography (HPGPC), Fourier-transform infrared (FTIR) spectroscopy, and NMR spectroscopy. The *in vitro* antitumor activity of PL-N1 was also evaluated in this paper.

2. Materials and methods

2.1. Materials and chemicals

PL-N, a water-soluble, high molecular weight heteropolysaccharide, was isolated from alkaline extract (1.25 M NaOH/0.05% $NaBH_4$) of the cultured *P. linteus* KCTC6190 mycelia according to our previous study (Wang et al., 2014). Monosaccharide standards, DEAE-Sephadex A-25, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were of laboratory grade and used without further purification.

2.2. Isolation and purification of the polysaccharide PL-N1

PL-N (1.0 g) was dissolved in 20 mL deionized water, centrifuged, loaded on a pre-equilibrated column (1.5 cm × 100 cm) of DEAE-Sephadex A-25, and eluted successively at a flow rate of 2 mL/min with deionized water and a gradient of 0–1.0 M NaCl solution. Each fraction (10 mL) was collected and analyzed using the anthrone test. The leading peak with the highest polysaccharide content was eluted with deionized water, collected, concentrated, dialyzed, and lyophilized to yield one purified polysaccharide designated as PL-N1 (Fig. 1A.)

The total carbohydrate and protein contents of PL-N1 were determined by phenol–sulfuric acid and Bradford (Bradford, 1976; Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), respectively. Optical rotation was measured of the PL-N1 sample dissolved in deionized water at 20 °C on a Perkin-Elmer 341 digital polarimeter at 589 nm.

2.3. Homogeneity and molecular weight determination

The homogeneity and molecular weight of PL-N1 was determined by HPGPC, which was performed on a Waters 1515 isocratic pump and a Waters 2414 refractive index detector with two Ultrahydrogel™ columns 250 and 2000 in series. Deionized water was used as the solvent for PL-N1 (2 mg/mL) and the eluent. The flow rate was kept at 0.6 mL/min. The column temperature was maintained at 50 ± 0.1 °C. Dextran M_w standards ranging from 5.2 to 1482 kDa (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) were used for calibration. Data were collected and analyzed by the online Breeze software package (Waters Corp., Milford, MA, USA).

2.4. Monosaccharide composition analysis

The PL-N1 sample (5 mg) was hydrolyzed with 2 M H_2SO_4 at 100 °C for 8 h in a sealed test tube. The acid was removed by neutralization with $BaCO_3$. The sample was centrifuged and evaporated to dryness under reduced pressure. The hydrolysate was converted into the nitrile acetate, and GC analysis was performed under the conditions as previously reported (Yan, Li, Wang, & Wu, 2010).

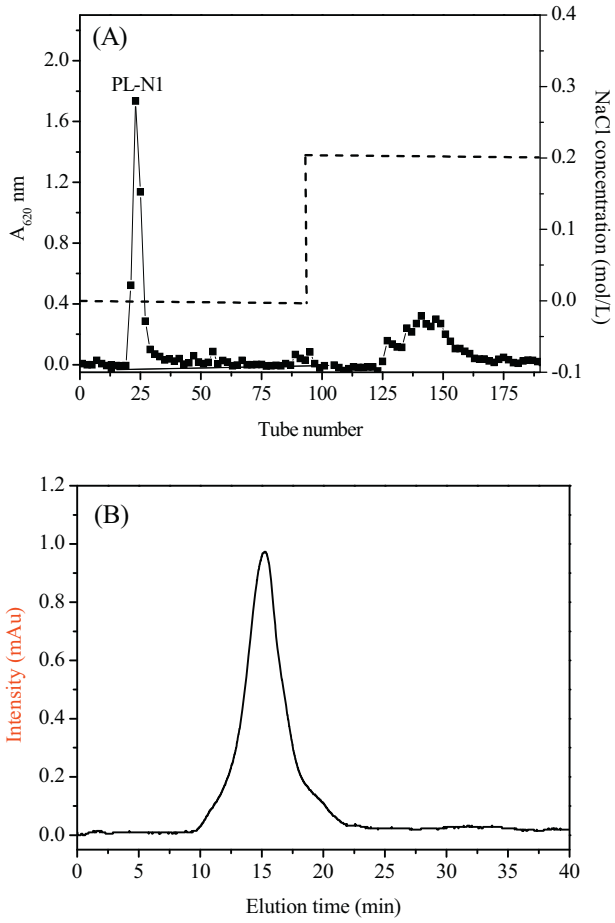

Fig. 1. (A) The purification of crude polysaccharides isolated from alkaline extract of cultured *P. linteus* mycelia by DEAE-Sephadex A-25 fast flow column chromatography; (B) The gel permeation chromatogram of PL-N1.

2.5. Methylation analysis

Freeze dried PL-N1 (10 mg) was methylated thrice using the (Needs & Selvendran, 1993) method with minor modifications. PL-N1 was weighed precisely and dissolved in 3.0 mL of dimethyl sulfoxide (DMSO), before quickly adding 200 mg of dried NaOH. After incubation for 30 min at room temperature (25 °C), 1.0 mL methyl iodide was added slowly to the reaction mixture solution. The mixture was incubated in darkness for 1 h. The remaining methyl iodide was decomposed using 2.0 mL deionized water. The methylated polysaccharide was extracted with 2 mL chloroform thrice and dried on a rotary evaporator at reduced pressure. Complete methylation was confirmed by the absence of hydroxyl peak in the infrared (IR) spectrum. The permethylated PL-N1 was depolymerized with 90% formic acid at 100 °C for 6 h and further hydrolyzed with 2 M trifluoroacetic acid at 100 °C for 4 h. The resulting hydrolysates were reduced with 20 mg NaBH4 and acetylated with acetic anhydride. The methylated alditol acetates were analyzed by GC-MS.

2.6. FTIR and NMR spectroscopies

The FTIR spectrum of the PL-N1 was determined using a Nexus 670 FTIR spectrometer (Thermo Nicolet Co., USA) in the wavenumber range from 500 to 4000 cm^{-1} with KBr pellets and referenced against air. PL-N1 (30 mg) was dissolved in 0.5 mL of D2O. The ^1H and ^{13}C NMR spectra were recorded by a Bruker AVANCEIII

Table 1
Preliminary structural characteristics of PL-N1.

Sample	Carbohydrate (%)	$[\alpha]_D^{20}$ (°)	M_w (kDa)	Monosaccharide composition (mol %)			
				Ara	Xyl	Glc	Gal
PL-N1	96.7 ± 1.5	−75 ± 1.0	343000	4.0	6.7	1.3	1.0

400 MHz spectrometer (Bruker, Rheinstetten, Germany) at 25 °C. All chemical shifts were expressed in reference to Me4Si.

2.7. Antitumor activity assay

The *in vitro* antitumor activity of PL-N1 was determined by MTT colorimetry (Jing et al., 2014; Zhu et al., 2013). A549, Bel7402, HCT-8, and HepG2 cells were cultured in RPMI 1640 culture solution containing 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a humidified atmosphere of 5% CO2 at 37 °C. The cell suspension (1.5 × 104 cells/mL) was incubated into 96-well plate (100 μL/well) for 24 h. Then, 100 μL of PL-N1 was added into each well with a concentration gradient of 50, 100, and 200 μg/mL. A total of 5-Fu (5 μg/mL) was used as the positive control. After incubation for 72 h, 100 μL of MTT (0.5 mg/mL) was added to each well, and the cells were incubated for another 4 h at 37 °C. Afterward, 150 μL of DMSO was added to each well. Absorbance was measured at 544 nm by enzyme-linked immunosorbent analyzer. The inhibition rate of cell growth was calculated according to the following formula:

$$\text{Inhibition rate}\,(\%) = \left(\frac{1 - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100. \quad (1)$$

3. Results and discussions

3.1. Preliminary properties and chemical composition of PL-N1

The crude polysaccharide (PL-N) was purified on a DEAE-Sephadex A-25 fast flow column chromatography with deionized water and different stepwise of NaCl solutions (0.2–1.0 M), leading to two fractions PL-N1 and PL-N2, PL-N1 was the leading polysaccharide fraction with a percentage of approximately 90% of PL-N (Fig. 1A). The main fraction PL-N1 with a yield of 35.1% based on the dried crude polysaccharide PL-N was collected, dialyzed, and lyophilized. PL-N1 was used for further structure characterization and biological activity.

The total sugar content of PL-N1 was estimated at 98.7% by the phenol–sulfuric acid method. PL-N1 had a negative response to the Bradford method. No absorption was observed at 280 nm in the UV spectrum, which also indicates the absence of protein. PL-N1 showed a single and symmetrical peak on the profile of HPGPC (Fig. 1B), which indicates its property as a homogeneous polysaccharide. The weight average molecular weight (M_w) of PL-N1 was estimated at 343,000 kDa based on the calibration equation, which was derived by linear regression of the calibration curve generated using the dextran M_w standards. PL-N1 was completely hydrolyzed by H_2SO_4 into individual monosaccharides that were derivatized into nitrile acetates for GC analysis. The results are listed in Table 1. Monosaccharide composition analysis demonstrated that PL-N1 comprised arabinose (Ara), xylose (Xyl), glucose (Glc), and Gal (Gal) in the molar ratio of 4.0:6.7:1.3:1.0. The optical rotation of PL-N1 was $[\alpha]_D^{20} = -75^\circ \pm 1.0^\circ$ (c 0.2, H_2O), which indicated that PL-N1 may mainly contain β-type glycosidic linkages in its sturcutre. These results show that PL-N1 is a high molecular weight water-soluble heteropolysaccharide.

The FTIR spectrum of PL-N1 is shown in Fig. 2 Two characteristic absorptions of polysaccharides were observed: a strong and wide stretching peak at approximately 3410 cm^{-1} for the O—H stretching vibration and a weak absorption peak of approximately 2910 cm^{-1} for the C—H stretching vibration. The relatively strong and the weak absorption peaks at 1618 and 1438 cm^{-1}, respectively, also suggest the characteristic IR absorption of polysaccharides (Ge, Zhang, & Sun, 2009). The peak at 1040 cm^{-1} was attributed to the C—O bands in the IR spectrum. No absorption peak was observed at 1740 cm^{-1}, which indicates the absence of uronic acids in the polysaccharide structure (Jing et al., 2014). This result further suggests that PL-N1 is a neutral polysaccharide.

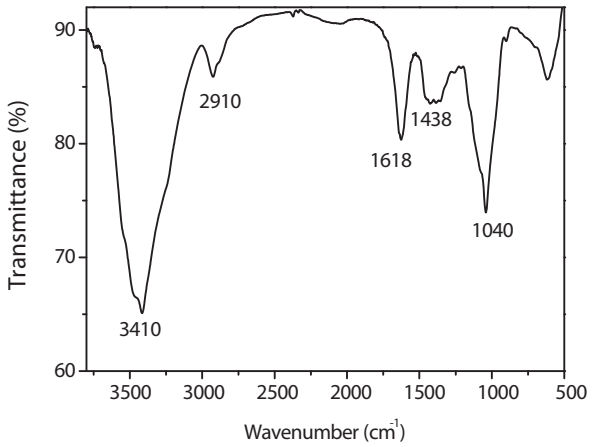

Fig. 2. The FT-IR spectrum of PL-N1.

3.2. Structural characterizations of PL-N1

Methylation analysis was employed to identify the interglycosidic linkages between the monosaccharide residues of PL-N1. The methylated polysaccharide was subjected to acid hydrolysis, reduction, and acetylation. The resulting partially methylated alditol acetates (PMAA) were analyzed by GC-MS. The linkage patterns of PL-N1 are summarized in Table 2 according to relative literature and the standard data from the Complex Carbohydrate Research Center (University of Georgia, USA) Spectral Database for PMAA (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005; Wang, He, & Huang, 2007). The results show that PL-N1 comprised seven glycosidic residues, i.e., Araf (1→, →5) Araf (1→, →4) Xylp (1→, →2) Xylp (1→, →2,4) Xylp (1→, →4) Glcp (1→, and →4,6) Galp (1→ in the molar ratio of 2.28:1.83:3.17:2.64:1.03:1.36:1.00. This result suggests that the number of branched residues in polysaccharide structure were equal to the number of terminal units. The molar ratios were also consistent with the monosaccharide composition of PL-N1.

The fine chemical structures of PL-N1 including sugar compositions, configurations, and types of glycosidic linkages were further elucidated by NMR spectroscopy. All ^1H and ^{13}C signals are assigned and summarized in Table 3 based on the data available in literature (Agrawal, 1992; Zhang, 1999) and combinations with the ^1H and ^{13}C NMR experiments (Fig. 3). The resonances in the region of 97.0–109.0 ppm in ^{13}C NMR spectrum of PL-N1 were attributed to the anomeric carbon atoms of D-arabinose, D-xylose, D-galactose, and D-glucose. The peaks at 97.76 corresponding to C-1 of (1 → 4)-linked α-D-Glc unit, 99.67 corresponding to

Table 2
GC-MS results of methylated products of PL-N1.

Methylation product	Retention time (min)	Mass fragments (m/z)	Linkage type	Molar ratio
2,3,5-tri-O-Me-Araf	10.38	43,71,87,101,117,129,145,161	Araf(1→	2.28
3,4-di-O-Me-Xylp	13.93	43,71,87,101,117,129,161	→2)Xylp(1→	2.64
2,3-di-O-Me-Xylp	15.58	43,87,101,117,129,189	→4)Xylp(1→	3.17
2,3-di-O-Me-Araf	19.38	43,71,87,101,117,129,161	→5)Araf(1→	1.83
3-O-Me-Xylp	19.47	43,87,129,189	→2,4)Xylp(1→	1.03
2,3,6-tri-O-Me-Glcp	21.19	43,87,101,113, 117,129,131,233	→4)Glcp(1→	1.36
2,3-di-O-Me-Galp	26.45	43,87,99,101,117,129,161,201,261	→4,6)Galp(1→	1.00

Table 3
^{1}H and ^{13}C NMR chemical shifts of PL-N1 recorded in D_2O at 25 °C.

Sugar residue	Chemical shifts (ppm)					
	C-1/H-1	C-2/H-2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H-6
A α-D-Araf(1→	108.67/5.10	82.11/4.06	77.32/3.72	84.34/3.96	62.95/3.57	
B →5)-α-D-Araf(1→	107.92/4.90	81.37/4.03	76.72/3.72	83.18/4.07	72.38/3.52	
C →2,4)-β-D-Xylp(1→	102.43/4.42	75.83/4.00	74.27/3.63	76.82/3.50	62.65/4.12	
D →4)- β-D-Xylp(1→	101.68/4.36	72.81/3.16	75.56/3.47	76.37/3.72	61.26/4.07	
E →4,6)-β-D-Galp(1→	101.26/4.97	73.36/4.02	75.58/3.63	82.76/3.81	76.92/3.72	70.71/4.01
F →2)-α-D-Xylp(1→	99.67/5.27	72.71/3.54	73.65/3.83	69.41/3.85	63.06/4.03	
G →4)-α-D-Glcp(1→	97.76/5.16	71.27/3.63	72.95/3.80	81.45/3.82	71.56/3.93	60.49/3.55

C-1 of (1 → 2)-linked α-D-Xyl unit, 101.26 corresponding to C-1 of (1 → 4,6)-linked β-D-Gal unit, 101.68 corresponding to C-1 of (1 → 4)-linked β-D-Xyl unit, 102.43 corresponding to C-1 of (1 → 2,4)-linked β-D-Xyl unit, 107.92 corresponding to C-1 of (1 → 5)-linked α-D-Ara unit, and 108.67 corresponding to C-1 of (1→)-linked α-D-Ara unit. The ^{1}H NMR spectrum contained seven signals at 5.27, 5.16, 5.10, 4.97, 4.90, 4.42, and 4.36 ppm for the anomeric protons, suggesting that seven sugar residues, designated (1 → 2)-linked α-D-Xyl, (1 → 4)-linked α-D-Glc, (1→)-linked α-D-Ara, (1 → 4,6)-linked β-D-Gal, (1 → 5)-linked α-D-Ara, (1→2,4)-linked β-D-Xyl, and (1 → 4)-linked β-D-Xyl units, respectively, were present in PL-N1 (Table 3). From the profile of ^{13}C NMR spectrum, the main →4)- β-D-Xylp-(1→ linkage was characterized by five strong signals at 101.68, 72.81, 75.56, 76.37, and 61.26 ppm; while the →2)-α-D-Xyl-(1→ and →2,4)-β-D-Xylp(1→ linkages were characterized by signals at 99.67, 72.71, 73.65, 69.47, 63.06, and 102.43, 75.83, 74.27, 76.82, 62.65 ppm, respectively. All of them originated from C-1, C-2, C-3, C-4, and C-5 of the D-xylofuranosyl unit. Similarly, α-D-Araf (1→, →5)-α-D-Araf(1→,→4,6)-β-D-Galp(1→, and →4)-α-D-Glcp(1→ linkages could be also assigned according to their chemical shifts in the ^{13}C NMR spectrum (Table 3). These results also indicated that the backbone was composed of (1 → 2,4)-linked β-D-xylofuranosyl and (1 → 4,6)-β-D- -galpyranosyl residues, which branched at O-2 and O-6, respectively.

Based on the results of monosaccharide composition, methylation analysis, FTIR, and NMR measurements, we conclude that heteropolysaccharide PL-N1 has a backbone of (1 → 4)-linked β-D-xylopyranosyl residues, (1 → 2)-linked α-D-xylopyranosyl residues, (1 → 4)-linked α-D-glucopyranosyl residues, (1 → 5)-linked β-D-arabinofuranosyl residues, (1 → 4)-linked β-D-xylopyranosyl residues which branched at O-2, and (1 → 4)-linked β-D-galactopyranosyl residues which branched at O-6. The branches consisted of (1→)-linked α-D-arabinofuranosyl residues. The possible chemical structure of the repeating unit of PL-N1 is shown in Fig. 4.

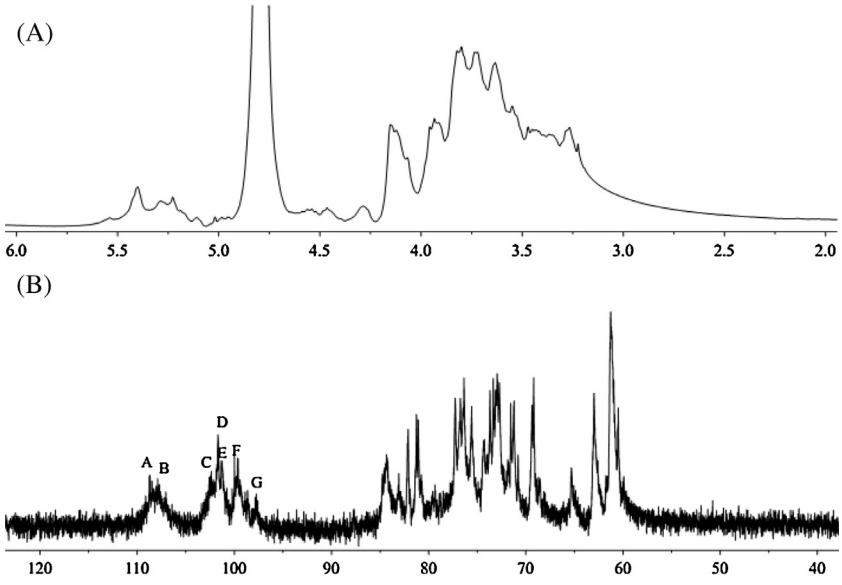

Fig. 3. NMR spectra of PL-N1. (A) ^{1}H spectrum; (B) ^{13}C spectrum.

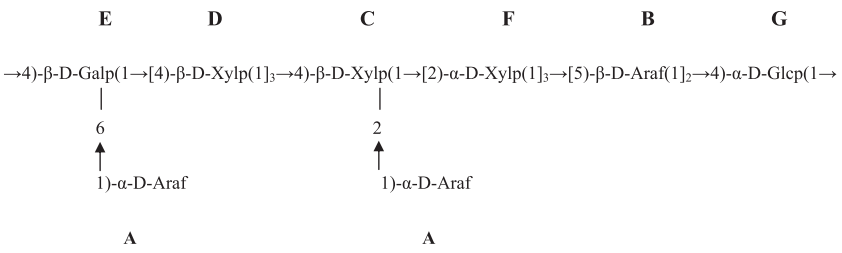

Fig. 4. Predicted structure of the repeating unit of PL-N1.

3.3. Polysaccharides with different structures found in Phellinus species

In the past two decades, a variety of polysaccharides with different monosaccharide constituents, molecular weights and chemical structures have been isolated from *P. linteus* and other *Phellinus* species. Kim, Park, Nam, Lee, & Lee (2003) reported a pure acidic proteo-heteroglycan with an average molecular weight of 150 kDa isolated from the fruiting body of *P. linteus* by hot water extraction, which contained 72.2% polysaccharide and 22.3% protein. It was a novel biomolecule mixed both α- and β-linkages, and a (1, 6) branched type (1, 3) glycan. The acidic proteo-heteroglycan exhibited significant immunomodulating and antitumor activity. Baker, Kim, & Park, 2008) characterized a water-soluble glycan isolated from a hot water extract of *P. linteus* having an average M_w of 25 kDa. It had a structure of a core β (1–3) linked glucan heavily substituted via (1–6) links with β (1–3) linked mannose chains, which was a common feature of mushroom glycans possessing antitumor activity. Yang et al., 2007; Yang, Ye, Zhang, Liu, & Tang, 2009 isolated two heteropolysaccharides PIP60-1 and PIPS1 from the hot water extract of *P. igniarius* fruiting bodies with molecular weights of 17.1 and 22 kDa, respectively. PIPS1 composed of fucose, galactose, mannose, and 3-O-Me-galactose, and it differed from PIP60-1, in lacking a glucose component. Both PIPS1 and PIP60-1 were structurally unique compared to polysaccharides previously isolated from *Phellinus* species. PIPS1 could stimulate the proliferation of spleen lymphocytes. Up to now, however, the previous studies on *Phellinus* polysaccharides almost isolated from hot water extracts of the fruiting bodies (Chen et al., 2011; Ge et al., 2009, 2013; Kim et al., 2003; Liu and Wang, 2007). In this work, PL-N1 was a novel heteropolysacharide isolated from an alkaline extract of the cultured *P. linteus* mycelia with a high molecular weight.

3.4. Antitumor activity of PL-N1

Four cancer cell lines, namely, A-549, Bel7402, HCT-8, and HepG2, were chosen to evaluate the antitumor potential of PL-N1 *in vitro* using the MTT method. All tested cells were treated with PL-N1 at 50 μg/mL and incubated for 72 h. PL-N1 exhibited no or few cytotoxicity on A-549, Bel7402, and HCT-8 cells (Fig. 5A). However, PL-N1 exhibited approximately 13% inhibition effect against HepG2 cells at 50 μg/mL, which indicates that PL-N1 possess a potent antitumor effect on HepG2 cells *in vitro*. Furthermore, PL-N1 exhibited a dose-dependent behavior within the dose range of 50–200 μg/mL. The inhibition effects of PL-N1 on HepG2 cells increased from 12.7 to 24.0% along with the increased sample concentration (Fig. 5B). The highest inhibition rate on HepG2 cancer cells was 24.0% at 200 μg/mL. Thus, PL-N1 appears to be capable of exerting potential inhibition effects on HepG2 cell proliferation.

Fungal polysaccharides that modulate the host biological responses against tumors have been confirmed and developed for applications in cancer therapy and functional food (Giavasis, 2014; Ooi and Liu, 2000; Zhang et al., 2007). The antitumor activity of polysaccharides can be influenced by their monosaccharide composition, protein contents, molecular mass, and chain conformation (Zhang et al., 2007). Generally, greater molecular mass and higher water solubility of polysaccharides indicate higher antitumor activity. The antitumor assay in this study showed that, to a certain extent, PL-N1 exhibits antitumor activity against the growth of HepG2 cells. This antitumor activity manifests a water-soluble heteropolysaccharide with a M_w of approximately 343,000 kDa, which contains Ara, Xyl, Glc, and Gal. Yan, Yin, Zhang, Yang, & Yu (2013) reported that a novel polysaccharide (TMP70 W) from *Taxus yunnanensis*, which was composed of Ara and Gal, exhibited inhibitory activity against K562 and MCF-7 cells. Ara and Gal were also present in PL-N1, which may be responsible for its antitumor activity. In addition, the level of antioxidation and reactive oxygen

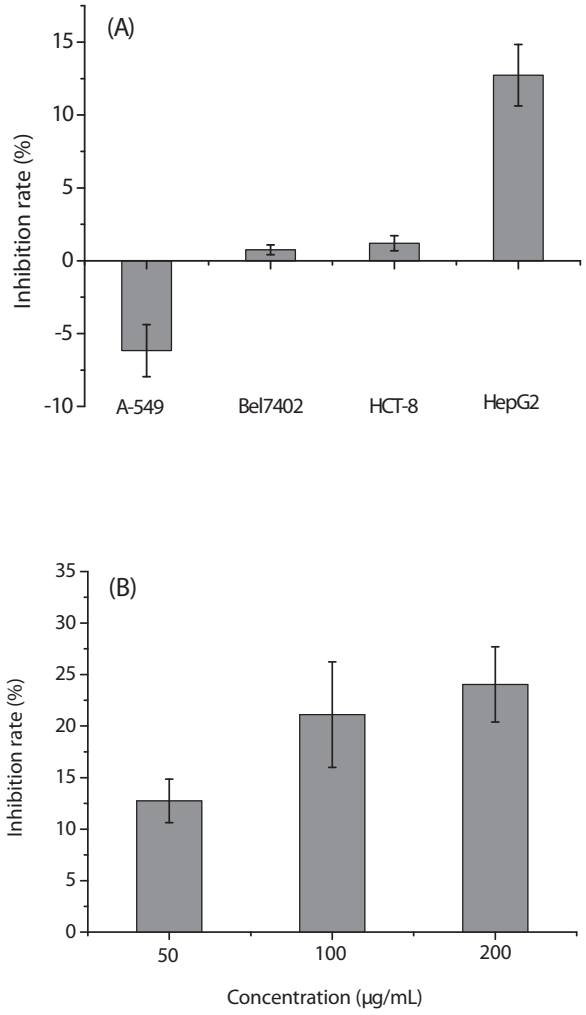

Fig. 5. *In vitro* antitumor activity of PL-N1. (A) Inhibitive effects of PL-N1 on A-549, Bel7402, HCT-8, and HepG2 cells at 50 μg/mL; (B) Inhibitive effects of PL-N1 on HepG2 cells with different concentrations (50, 100, 200 μg/mL).

species has an important influence on the generation and malign transformation of cancer cells (Xie et al., 2013). A polysaccharide (PL-N) obtained from alkaline extract of cultured *P. linteus* mycelia with strong antioxidant activity and radical-scavenging capacity *in vitro* was found in our previous study (Wang et al., 2014). In the present study, the *in vitro* antitumor activity of PL-N1 purified from PL-N may be partially attributed to its relatively stronger antioxidant activities.

4. Conclusions

In summary, a new water-soluble heteropolysaccharide (PL-N1) with a M_w of 343,000 kDa was obtained from cultured *P. linteus* mycelia through alkaline extraction. The detailed structural characterizations of PL-N1 were elucidated by a combination of physicochemical and instrumental analyses, such as acid hydrolysis, methylation analysis, GC, GC-MS, FTIR, and NMR. PL-N1 is a novel, neutral heteropolysaccharide found in *Phellinus* polysaccharides. The antitumor assay indicated that PL-N1 possesses potential antitumor activity against the growth of HepG2 *in vitro*. Therefore, PL-N1 could be explored as a new therapeutic agent and as a functional food. Further investigation on the possible mechanism of the antitumor activity of PL-N1 is suggested.

Acknowledgements

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