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Production, preliminary characterization and antitumor activity *in vitro* of polysaccharides from the mycelium of *Pholiota dinghuensis* Bi

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ABSTRACT

The production, preliminary characterization and antitumor activity *in vitro* of mycelial polysaccharides from *Pholiota dinghuensis* Bi (PDP) were investigated in the present study. Firstly, crude PDP was prepared from the mycelia of *P. dinghuensis* Bi by submerged culture. Then, the crude PDP was purified by chromatography of DEAE-52 cellulose and Sephadex G-100, resulting three purified fractions of PDP-1, PDP-2 and PDP-3. We found that the monosaccharide composition of PDP-3 was greatly different from that of PDP-1 or PDP-2. In addition, it contained the highest contents of protein, sulfate and uronic acid among the polysaccharides tested. Furthermore, PDP-3 exhibited higher antiproliferative activity against human gastric cancer BGC-823 cells *in vitro* than crude PDP, PDP-1 or PDP-2. At a concentration of 400 mg/L and an exposure time of 72 h, the inhibition rates for crude PDP, PDP-2 and PDP-3 were 69.36%, 73.65% and 85.78%, respectively.

1. Introduction

For thousands of years, mushrooms have been valued as an edible and medical resource. Recently, a number of bioactive molecules have been isolated and identified from mushrooms (Wasser, 2002; Zaidman, Yassin, Mahajna, & Wasser, 2005). Furthermore, it has been demonstrated that many higher basidiomycetes mushrooms contain bioactive polysaccharides in the fruit bodies, cultured mycelium and cultured broth (Chen, Zhao, Chen, & Li, 2008; Luo et al., 2010; Moradali, Mostafavi, Ghods, & Hedjaroude, 2007; Peng, Zhang, Zeng, & Kennedy, 2005; Zhang, Cui, Cheung, & Wang, 2007; Zhao, Dong, Chen, & Hu, 2010). As a vast and yet largely untapped source of powerful new pharmaceutical products, therefore, more attention has been paid to mushroom polysaccharides (Zhang et al., 2007).

Pholiota is a genus of small to fleshy mushroom in the family Strophariaceae. This genus has a widespread distribution, especially in temperate region. It has been reported that *Pholiota* is rich in vitamins, amino acids, trace elements, lipid and polysaccharides, and many of them represent various biological activities, such as anti-inflammatory, antioxidant, antitumor and hypolipidemic effects (Cui & Li, 2004; Li, Lu, Zhang, Lu, & Liu, 2008; Li & Wang, 2007; Li, Zhang, & Ma, 2010). *Pholiota dinghuensis* Bi was firstly reported in 1985 and only found in Guangdong Province, China (Bi, Li, Zheng, & Li, 1985; Kirk, Cannon, Minter, & Stalpers, 2008). As a specific kind of mushroom, its bioactive polysaccharides should be paid more attention. However, little information is available compared with those of other *Pholiota* mushrooms.

Therefore, we report here the production, purification, preliminary characterization and antitumor activity *in vitro* of the mycelial polysaccharides from *P. dinghuensis* Bi (PDP). Firstly, the effects of culture medium, temperature, inoculum volume and shaking speed on the production of PDP were investigated in the present study. Then, the crude PDP was sequentially purified by DEAE-52 cellulose ion-exchange chromatography and Sephadex G-100 size-exclusion chromatography. In addition, the crude PDP and its purified fractions were preliminary characterized by gas chromatography (GC) and Fourier-infrared spectroscopy (FT-IR). Finally, the antitumor activities *in vitro* of crude PDP and its purified fractions were determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

2. Materials and methods

2.1. Materials and reagents

P. dinghuensis Bi used in the present study was supplied by Guangdong Institute of Microbiology (Guangzhou, China). Human gastric cancer BGC-823 cells were obtained from the Cell Bank of Shanghai Institute of Cell Biology (Shanghai, China) and maintained

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in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) in an incubator (Thermo Fisher Scientific Inc., MA, USA) with a humidified 5% CO_2 at 37 °C.

Arabinose, fucose, galactose, glucose, mannose, MTT, penicillin, rhamnose, streptomycin and xylose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). FBS and RPMI-1640 media were purchased from Gibco/Invitrogen (Grand Island, NY, USA). All other reagents were of analytical grade.

2.2. Preparation of *P. dinghuensis* Bi polysaccharides

2.2.1. Culture of *P. dinghuensis* Bi

The *P. dinghuensis* Bi strain was maintained on potato dextrose agar (PDA) slant at 4 °C, and sub-cultured every two months. The seed culture was grown in a 250 mL flask containing 100 mL of seed medium (glucose 20 g/L, peptone 3 g/L, yeast extract 4 g/L, KH_2PO_4 1 g/L, and $MgSO_4$ 1 g/L, distilled water) at 25 °C without shaking for 12 h, and then with a shaking of 140 rpm for 4 days. For submerged culture, it was performed in 250 mL flasks containing 85 mL of fresh medium and 15 mL of seed culture at 25 °C on a rotary shaker (140 rpm) for 5 days. The basal media for the submerged cultivation contained: SCS (synthetic carbon source, composed by glucose and corn starch) 40 g/L, SNS (synthetic nitrogen source, composed by peptone and yeast extract) 4 g/L, KH_2PO_4 1 g/L, $MgSO_4$ 1.5 g/L, and distilled water.

2.2.2. Preparation of crude PDP

The crude PDP was prepared according to the reported method with some modifications (Luo et al., 2010). Briefly, the mycelia of *P. dinghuensis* Bi were harvested from the liquid medium by filtering through a filter paper, washed three times with distilled water, and dried at 60 °C to a constant weight. The dry weight (DW) of the mycelia was determined in terms of g/L. The dry mycelia were ground using a domestic blender, extracted three times with distilled water at 90 °C for 2 h each, and then centrifuged at 5000 rpm for 20 min. The supernatants were combined and concentrated to one-fifth of its original volume with a rotary evaporator under reduced pressure. The resulting concentrate was mixed with three times volume of absolute ethanol, stirred vigorously and kept overnight at 4 °C. The precipitate was collected by centrifugation at 5000 rpm for 20 min, washed twice with acetone and ether, respectively, and then dried. The resulting crude PDP was also estimated in terms of g/L (DW).

2.2.3. Purification of crude PDP

The crude PDP was purified sequentially by chromatography of DEAE-52 and Sephadex G-100 according to our reported method (Qiao et al., 2009). Briefly, 5 mL of crude PDP solution (10 mg/mL) was applied to a column of DEAE-52, and the column was stepwise eluted with 0, 0.1, 0.3 and 0.5 M sodium chloride solutions at a flow rate of 60 mL/h. Eluate was collected automatically (5 mL/tube), and the carbohydrates were determined by the phenol–sulfuric acid method using glucose as the standard (Dubios, Gilles, Hamilton, Rebers, & Smith, 1956). As results, three fractions of polysaccharides (F-1, F-2 and F-3) were obtained, concentrated, dialyzed against distilled water and further purified through a column of Sephadex G-100, affording PDP-1, PDP-2 and PDP-3, respectively. The three purified fractions were collected, concentrated, dialyzed and lyophilized for further study, respectively.

2.3. Preliminary characterization of PDP

2.3.1. Determination of contents of carbohydrate, sulfuric radical, protein and uronic acid

The contents of carbohydrate, protein and uronic acid were determined according to the reported methods (Blumenkrantz & Asboe-Hansen, 1973; Bradford, 1976; Dubios et al., 1956), using glucose, bovine serum albumin and glucuronic acid as the standards, respectively. The content of sulfate in polysaccharide was measured by the method of barium chloride–gelatin (Lloyd, Dodgson, Price, & Rose, 1961).

2.3.2. Analysis of monosaccharide composition

The monosaccharide compositions of crude PDP and its purified fractions (PDP-1, PDP-2 and PDP-3) were determined using the method reported by Qiao et al. (2009) with slight modification. Briefly, the polysaccharide sample (5.0 mg) was hydrolyzed with 4 mL trifluoroacetic acid (TFA, 2 M) at 120 °C in an oven for 2 h, and the excess TFA was removed by evaporation at a temperature of 40 °C. Then, the hydrolyzate was repeatedly co-concentrated with methanol to dryness and acetylated by the addition of a mixture of methanol, pyridine and acetic anhydride. The monosaccharide standards including rhamnose, arabinose, fucose, xylose, mannose, glucose and galactose were acetylated in the same way. Finally, the acetylated samples were analyzed by a 6890N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detector and a HP-5 fused silica capillary column (30 m × 0.32 mm × 0.25 mm). The oven temperature was maintained at 120 °C for 3 min, and then increased gradually to 210 °C at a rate of 3 °C/min. The temperatures of detector and injector were set at 280 °C and 250 °C, respectively. The flow rates of N_2, H_2 and air were 25, 30 and 400 mL/min, respectively.

2.3.3. Infrared spectral analysis

FT-IR analyses of crude PDP, PDP-1, PDP-2 and PDP-3 were carried out by the potassium bromide (KBr) pellet method with a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific Inc., MA, USA) in the range of 400–4000 cm^{-1}.

2.4. Assay of inhibition activity in vitro on BGC-823 cell proliferation

Inhibition activities *in vitro* of crude PDP and its three purified fractions on human gastric cancer BGC-823 cell proliferation were evaluated as described by Mosmann (1983) using MTT-based colorimetric method. Briefly, BGC-823 cells in a density of 1×10^5 cells/mL in the RPMI-1640 medium supplemented with FBS (10%), penicillin (100 U/mL) and streptomycin (100 μg/mL) were pipetted into 96-well flat-bottom plate (100 μL/well). After 12 h of incubation at 37 °C in a humidified 5% CO_2 incubator, non-adherent cells were removed by washing three times with RPMI-1640 medium. Then, fresh medium (100 μL/well, control group) or test sample (100 μL/well, crude or purified PDP at a final concentration of 5, 10, 20, 50, 100, 200 and 400 mg/L) was added to each well, and the cells were incubated for 24, 48 and 72 h, respectively. After incubation, MTT solution (10 μL/well, 5 mg/mL) was added to each well, and the plate was incubated for an additional 4 h at 37 °C. Finally, 100 μL of 10% SDS in 0.01 N HCl was added to each well and the plate was kept overnight for the dissolution of formazan crystals. The absorbance of each well at 570 nm was measured by an ELISA plate reader (BioTek Instruments Inc., Winooski, VT, USA). The inhibition rate was calculated according to the formula below:

$$\text{Inhibition rate } (\%) = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

Table 1
Effects of carbon sources and nitrogen sources on the cell biomass and crude PDP production by *P. dinghuensis* Bi.

Item	Dry mycelium weight	Crude PDP
Carbon source (4%)		
Fructose	$3.405 \pm 0.241^{c,d}$	0.283 ± 0.014^{c}
Glucose	$3.695 \pm 0.274^{d,e}$	0.349 ± 0.011^{d}
Lactose	2.523 ± 0.202^{b}	0.209 ± 0.022^{b}
Maltose	3.333 ± 0.319^{c}	0.277 ± 0.013^{c}
Sucrose	2.577 ± 0.193^{b}	0.214 ± 0.021^{b}
Xylose	1.064 ± 0.283^{a}	0.088 ± 0.007^{a}
Corn starch	$3.951 \pm 0.208^{e,f}$	0.358 ± 0.019^{d}
Synthetic carbon source	4.248 ± 0.302^{f}	0.399 ± 0.016^{e}
Nitrogen source (0.4%)		
Beef extract	2.288 ± 0.172^{b}	0.191 ± 0.007^{c}
Yeast extract	$3.434 \pm 0.127^{c,d}$	0.316 ± 0.012^{d}
Peptone	3.281 ± 0.313^{c}	0.297 ± 0.008^{d}
$(NH_4)_2SO_4$	1.942 ± 0.201^{b}	0.162 ± 0.011^{b}
$NaNO_3$	1.319 ± 0.162^{a}	0.110 ± 0.013^{a}
Synthetic nitrogen source	3.844 ± 0.351^{d}	0.340 ± 0.016^{e}

Different superscript alphabet letters in each column indicated significant differences at $p < 0.05$ level. Data were means ± SD of triplicates.

2.5. Statistical analysis

Data were analyzed by SPSS and expressed as mean ± standard deviation (SD) for at least three replicates. Significance was determined at $p < 0.05$ by analysis of variance (ANOVA) followed by Duncan's multiple comparison tests.

3. Results and discussion

3.1. Production of crude PDP

3.1.1. Effects of carbon source and nitrogen source on PDP production

In order to select the suitable carbon source for the mycelial growth and PDP production in *P. dinghuensis* Bi, various carbon sources were separately provided in place of SCS in the basal medium for the submerged cultivation. As shown in Table 1, among the carbon sources tested, SCS and corn starch yielded higher dry mycelium weights, 3.951 ± 0.208 and 4.248 ± 0.302 g/L, respectively. However, there was no significance ($p < 0.05$) between SCS and corn starch. For PDP production, the maximum production (0.399 ± 0.016 g/L) was obtained when SCS was used as the carbon source. The result indicated that the addition of corn starch in the basal media could improve the growth of mycelia and PDP production.

The effects of various nitrogen sources on the growth of mycelia and PDP production were also investigated in the present study. It was easy to find that yeast extract and peptone were better nitrogen sources than beef extract and inorganic nitrogen sources, and the combined use of yeast extract and peptone (SNS) yielded the highest biomass (3.844 ± 0.351 g/L) and PDP production (0.340 ± 0.016 g/L, Table 1). It might be due to that SNS as nitrogen source could provide more protein, amino acid, vitamins and some other growth factors for the cell growth and PDP production of *P. dinghuensis* Bi. Similar phenomenon was reported by other investigators (Liu et al., 2009; Pokhrel & Ohga, 2007).

3.1.2. Effects of inoculum volume, culture temperature and rotary speed on PDP production

To determine the optimal inoculum volume for biomass and PDP production, fermentation were done with different inoculum volumes in a range of 5–20% (v/v). As shown in Fig. 1A, significant increases of mycelial biomass and PDP production with the increase of inoculum volume from 5% to 12.5% were observed, and the highest yields of cell biomass and PDP production were recorded at an inoculum volume of 15%. However, there was no significant effect when the inoculum volume was over 15% ($p < 0.05$). To our knowledge, a higher inoculum volume could result a shorter culture time, but no more cell growth or PDP production would be obtained at the same time because of less nutrition and space for cells in the culture broth.

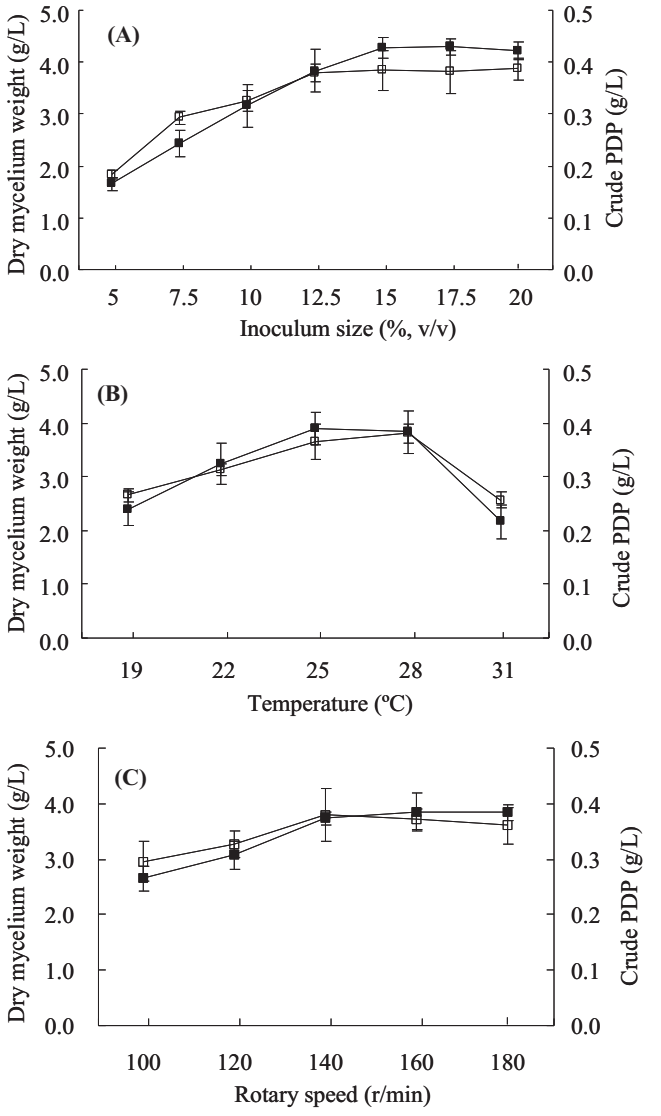

Fig. 1. Effects of inoculum size (A), culture temperature (B) and rotary speed (C) on the cell growth (□) and crude PDP production (■) by *P. dinghuensis* Bi. Data are means ± SD of triplicates.

The influence of culture temperature on the cell growth and PDP production of *P. dinghuensis* Bi is shown in Fig. 1B. The optimal culture temperature for PDP production was 25 °C, while higher or lower temperature resulted in less PDP production. The result suggested that *P. dinghuensis* Bi favored lower temperature for PDP production, which is comparable to many other kinds of mushrooms that favor relatively low temperature in their submerged cultures (Bae, Sinha, Park, Song, & Yun, 2000; Chen et al., 2008; Kim et al., 2005).

The effects of shaking speed on the cell growth and PDP production were also investigated. A marked improvement on PDP production was observed when an increase of rotary speed was applied during the culture (Fig. 1C). Notably, it favored the cell growth and PDP production at rotary speed of 140–160 rpm. In

general, oxygen for cell growth and its product synthesis is usually satisfied by increasing rotary speed. However, it might cause an increase of shear stress on the mycelium with a higher shaking speed. For saving energy, 140 rpm was considered suitable for the shaking speed in this study.

3.2. Purification of crude PDP

In the present study, the crude PDP was prepared from the mycelia of *P. dinghuensis* Bi by hot-water extraction, centrifugation and ethanol precipitation. The solution of crude PDP was loaded onto an anion-exchange chromatography column of DEAE-52 cellulose, and the column was stepwise eluted with 0, 0.1, 0.3 and 0.5 M sodium chloride solutions. Three independent elution peaks (F_1, F_2 and F_3, Fig. 2A) detected by the phenol–sulfuric acid assay were obtained. Then, the three fractions were collected, dialyzed, concentrated and further loaded onto a column of Sephadex G-100, respectively. As results, each fraction afforded one single elution peak (Fig. 2B–D), named as PDP-1, PDP-2 and PDP-3, respectively. The recovery rates for PDP-1, PDP-2 and PDP-3, based on the amount of crude HCPS used, were 26.91%, 30.18% and 3.93%, respectively.

3.3. Preliminary characterization of PDP

3.3.1. Contents of carbohydrate, protein, uronic acid and sulfate in PDP

Table 2 shows the contents of carbohydrate, protein, uronic acid and sulfate in crude PDP and its purified fractions. The carbohydrate contents in crude PDP, PDP-1, PDP-2 and PDP-3 were 86.95%, 97.97%, 94.46% and 85.58%, respectively. Among all the polysaccharides tested, PDP-3 contained the highest contents of protein, uronic acid and sulfate. The contents of protein in crude PDP, PDP-1, PDP-2 and PDP-3 were 1.46%, 0.23%, 0.69% and 4.03%, respectively. The high content of protein for PDP-3 suggested that it might be protein-bound polysaccharide (Peng et al., 2005).

3.3.2. Monosaccharide composition of PDP

The monosaccharide compositions of crude PDP and its purified fractions (PDP-1, PDP-2 and PDP-3) are presented in Fig. 3 and Table 2. Notably, glucose was found to be the most abundant monosaccharide, in a ratio of 92.54%, 92.24%, 88.65% and 67.83%, respectively, for crude PDP, PDP-1, PDP-2 and PDP-3. For crude PDP, it was composed of arabinose, fucose, xylose, mannose, glucose and galactose in relative percent of 2.18, 1.06, 1.48, 0.92, 92.54 and 1.82, respectively. However, the monosaccharide composition of PDP-3 was different from that of PDP-1 or PDP-2. Interestingly, xylose and rhamnose were found only to be present in PDP-3. In addition, the contents of fucose, mannose and galactose in PDP-3 were relatively higher than those in PDP-1 or PDP-2.

3.3.3. FT-IR analysis

The FT-IR spectra of crude PDP, PDP-1, PDP-2, and PDP-3 are depicted in Fig. 4. A strong and broad absorption peak at 3363 cm^{-1} for O–H stretching vibrations, a peak at 2927 cm^{-1} for C–H stretching vibrations, and a strong extensive absorption in the region of 900–1200 cm^{-1} for coupled C–O and C–C stretching and C–OH bending vibrations were observed in the polysaccharide samples, indicating the characteristic absorptions of polysaccharides (Liu et al., 2008). Furthermore, two strong bands at 1653 and 1414 cm^{-1} were assigned to the absorbance of the deprotonated carboxylic group (COO^-), indicating PDP-2 and PDP-3 be acidic polysaccharide (Zou, Zhang, Yao, Niu, & Gao, 2010), which is in good accordance with the analytical results for PDP-2 and PDP-3 (Table 2). Absorption peaks at 1240 cm^{-1} and 1154 cm^{-1} were assigned, respectively, to the asymmetric and symmetric stretching vibrations of S=O, an evidence of sulfate ester, confirming directly PDP-2 and PDP-3 were sulfated polysaccharides. What's more, the relative stronger absorption peak at 1653 cm^{-1} for N–H bending vibration might be related to the higher content of protein in PDP-3 (Qiao et al., 2009).

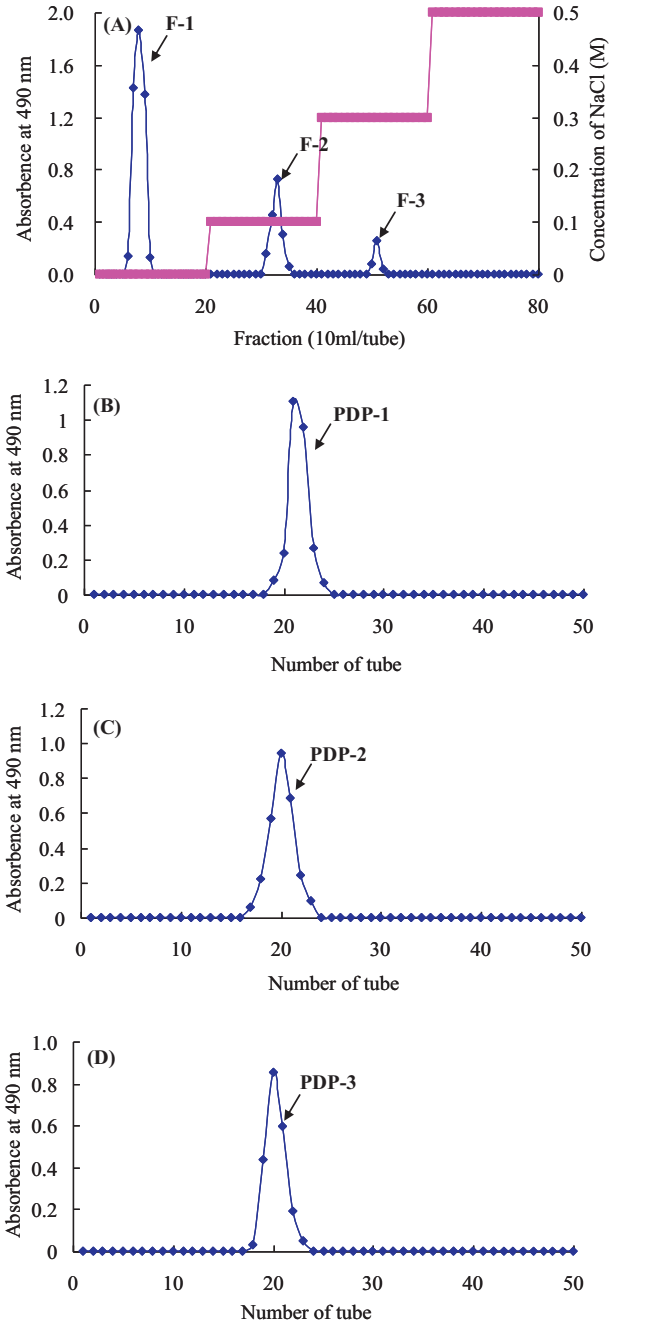

Fig. 2. Stepwise elution curve of crude PDP on DEAE-52 column (A) and elution curves of polysaccharides fractions (F-1, F-2 and F-3) on Sephadex G-100 column (B–D).

3.4. Antitumor activity in vitro of PDP

In the present study, the antiproliferative activities *in vitro* of crude PDP, PDP-1, PDP-2 and PDP-3 on the human gastric cancer BGC-823 cells were investigated. As shown in Fig. 5, all the polysaccharides exerted concentration-dependent inhibition effect on

Table 2
Preliminary characterization of crude PDP, PDP-1, PDP-2, and PDP-3.

Samples	Carbohydrate (%)	Uronic acid (%)	Protein (%)	Sulfuric radical (%)	Sugar component (%)						
					Rha	Ara	Fuc	Xyl	Man	Glc	Gal
Crude PDP	86.95	1.68	1.46	2.08	–[a]	2.18	1.06	1.48	0.92	92.54	1.82
PDP-1	97.97	1.14	0.23	1.17	–	–	2.04	–	1.83	92.24	3.90
PDP-2	94.46	1.75	0.69	2.26	–	–	2.28	–	6.01	88.65	3.08
PDP-3	85.58	2.31	4.03	3.81	1.75	–	4.35	7.67	11.11	67.83	7.30

[a] Not detected.

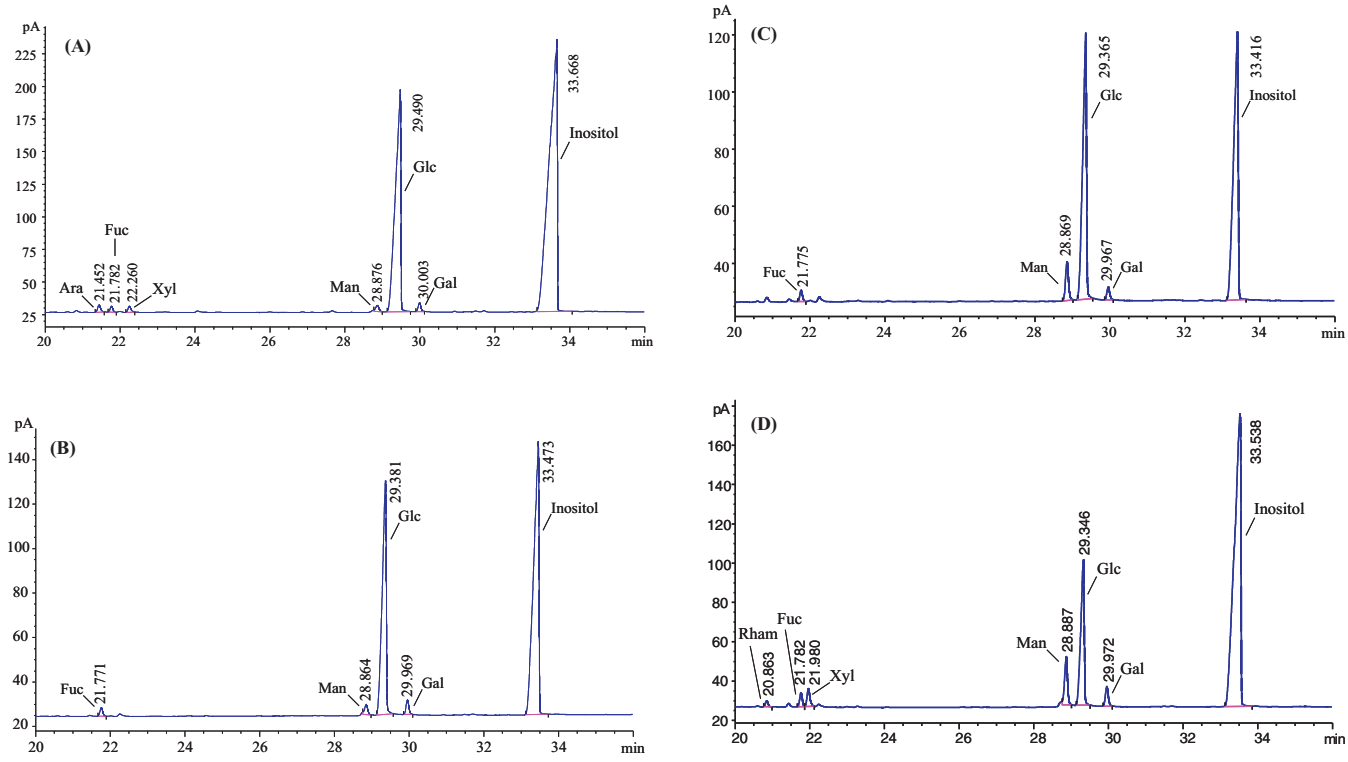

Fig. 3. GC chromatograms of crude PDP (A), PDP-1 (B), PDP-2 (C) and PDP-3 (D).

BGC-823 cells. After 24 h treatment, PDP-3 exhibited the strongest inhibition activity (inhibitory rate, 46.85%) at a dose of 400 mg/L, followed by PDP-2, crude PDP and PDP-1 with the corresponding inhibition rates of 42.79%, 35.17% and 16.13%, respectively. Furthermore, the effects of exposure time by crude PDP, PDP-1, PDP-2 and PDP-3 towards BGC-823 cells were investigated. Compared with those of 24 and 48 h exposure times, there were significant increases of inhibitory activity for 72 h exposure time. After 72 h incubation, the inhibition rates at a concentration of 400 mg/L for crude PDP, PDP-2 and PDP-3 were 69.36%, 73.65% and 85.78%, respectively (Fig. 5). However, the antiproliferative activity of PDP-1 was no more than 23.72% regardless the changes of dose and exposure time.

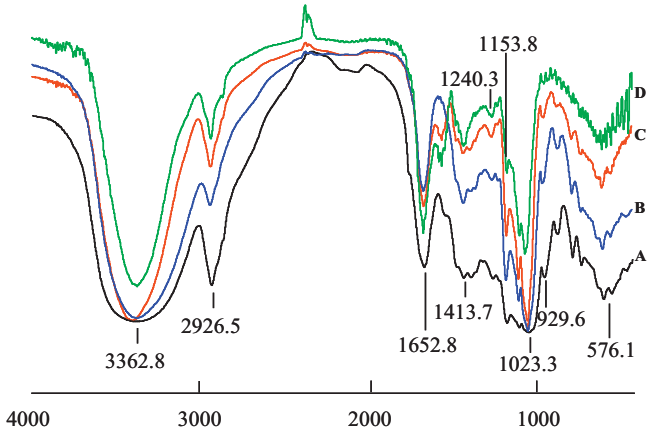

Fig. 4. FT-IR spectra of crude PDP (A), PDP-1 (B), PDP-2 (C) and PDP-3 (D).

It has been known for many years that selected mushrooms from a higher basidiomycetes origin are effective against cancers and low side-effects *in vivo* (Daba & Ezeronye, 2003; Frantz, 1989). Most reports confirmed that mushroom polysaccharides exerted their anti-tumor action via activation of the immune response of the host organism, and accordingly mushroom polysaccharides were regarded as biological response modifiers (Wasser, 2002; Zaidman et al., 2005). However, studies related with the effects of polysaccharides on the tumor cells have increased recently (Lee et al., 2003; Song & Du, 2010). It has also been reported that their antitumor effects depend on the molecular weight, chemical composition, structure of the polymeric backbone, degree of branching and so on (Cui et al., 2007; Jin et al., 2003). It is noteworthy that polysaccharides with high antitumor activity from the fruiting bodies of mushrooms are mostly heteropolysaccharides consisting of galactose, glucose, mannose and fucose, whereas those from the mycelia of mushrooms are mainly protein-containing glucans (Zaidman et al., 2005). In the present study, we found that PDP-3 possessed

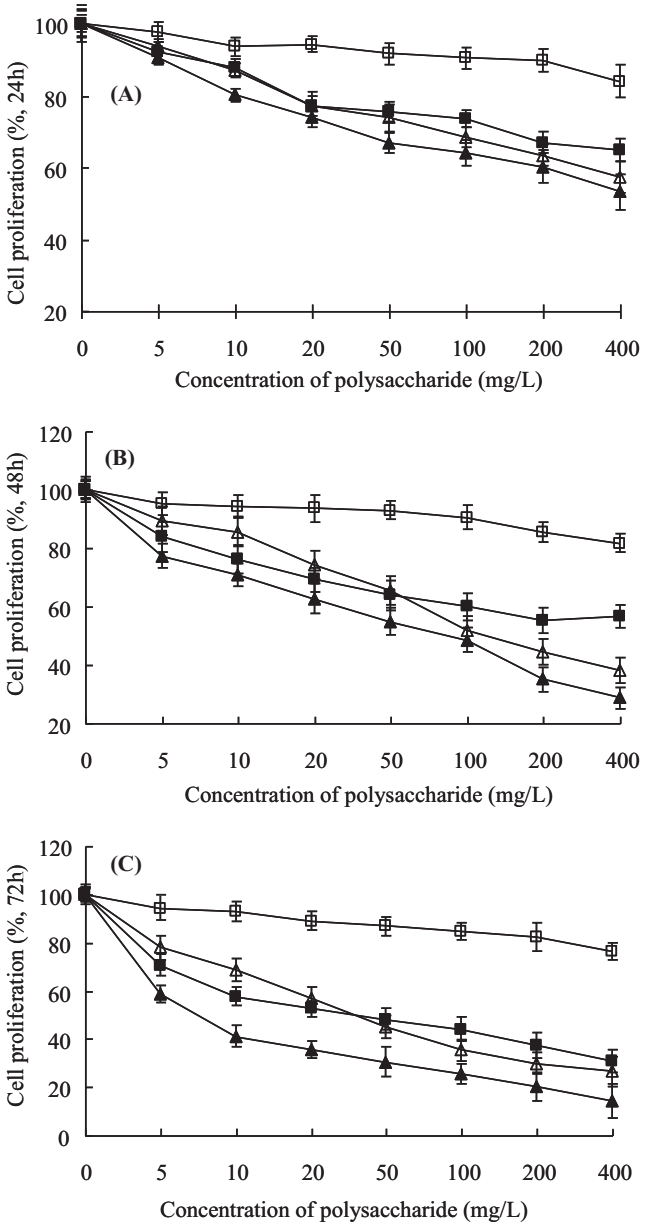

Fig. 5. The growth inhibition of crude PDP (■), PDP-1 (□), PDP-2 (△) and PDP-3 (▲) against human gastric cancer BGC-823 cells at 24 h (A), 48 h (B) and 72 h (C) treatment. Data are means ± SD of triplicates.

the highest antitumor activity *in vitro*, and PDP-2 exhibited significant higher antitumor activity than PDP-1. The differences of antitumor activity for PDP-1, PDP-2 and PDP-3 might be due to their differences in monosaccharide composition and contents of protein, sulfate and uronic acid. In the monosaccharide composition, PDP-3 contained only 68% glucose, but had higher ratios of fucose, rhamnose, xylose, mannose and galactose (Table 2). In addition, PDP-3 represented relative higher contents of protein, sulfate and uronic acid than PDP-1 or PDP-2. Comparing with PDP-1, PDP-2 contained a higher ratio of mannose (6.01%) in monosaccharide composition and relative higher contents of protein, sulfate and uronic acid. Accordingly, it exhibited stronger inhibitory effect than PDP-1. However, PDP-1 exhibited relative lower antitumor activity regardless of the treatment of time and polysaccharide concentration, and the reason might be due to its origin (the fraction eluted with water from the column of DEAE-52 cellulose during the purification of crude PDP), monosaccharide composition (mainly composed of glucose, 92%) and lower contents of protein, sulfate and uronic acid. The results demonstrate that the chemical property, monosaccharide composition and structure of mushroom polysaccharide play important roles in the antitumor activity of polysaccharide (Zaidman et al., 2005; Zou et al., 2010).

4. Conclusions

Little information about the production and biological activity of PDP has been documented in the literature. Therefore, the production, preliminary characterization and antitumor activity *in vitro* of PDP were investigated in the present study. Firstly, the crude PDP was obtained from the mycelia of *P. dinghuensis* Bi by submerged culture under a condition of SCS 40 g/L, SNS 4 g/L, inoculum volume 15% (v/v), culture temperature 25 °C and shaking speed 140 rpm. The crude PDP was then purified by chromatography of DEAE-52 cellulose and Sephadex G-100, and three fractions of PDP-1, PDP-2 and PDP-3 were obtained. We found that PDP-3 had the highest contents of protein, sulfuric radical and uronic acid among the polysaccharides tested. In addition, the monosaccharide composition of PDP-3 was greatly different from that of PDP-1 or PDP-2. Finally, we demonstrated that PDP-3 exhibited higher antitumor activity *in vitro* than crude PDP, PDP-1 and PDP-2, possibly due to their differences of the contents of protein, sulfuric radical, monosaccharide compositions and chemical structures. We speculate that the basic structure may be the key factor for activity, and further works are in progress.

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