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Purification, characterization and antitumor activity of polysaccharides from *Pleurotus eryngii* residue

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ABSTRACT

A novel water-soluble polysaccharide from *Pleurotus eryngii* residue was isolated and further purified by DEAE cellulose-52 chromatography and Sephadex G-100 size-exclusion chromatography to yield PEPE-1, PEPE-2 and PEPE-3. Molecular weights were determined by high-performance size-exclusion chromatography (HPSEC). Gas chromatography (GC) analysis of monosaccharide composition confirmed that PEPE-1, PEPE-2 and PEPE-3 were heteropolysaccharides and mainly composed of glucose. Sulfate and uronic acid content, ultraviolet and infrared spectrum were also evaluated. The antitumor activities of the polysaccharides against HepG-2 cells were studied *in vitro*. Results showed that the three polysaccharides could suppress the proliferation and enhance lactate dehydrogenase (LDH) release of HepG-2 cells in a dose- and time-dependent manner. The effect increased in the order of PEPE-1 < PEPE-2 < PEPE-3, respectively. The same order was also observed with uronic acid content. Findings presented in this study suggested that the polysaccharides extracted from *P. eryngii* residue might be suitable for functional foods and natural antitumor drugs development.

1. Introduction

For thousands of years, there has been increasing interest in the use of edible mushrooms as a source of natural medicine and functional food (Alam et al., 2009). Recently, *Pleurotus eryngii*, a new and an important species of edible mushrooms commonly known as the king oyster mushroom, has been successfully accepted among consumers in North Africa, Europe and Asia owing to its good taste and high nutritional value (Jeong, Jeong, Gu, Islam, & Song, 2010). *P. eryngii* may have multiple biological functions due to its unique varieties of bioactive compounds which are physiologically beneficial, such as monoterpene (Liu, Li, & Liu, 2013), polysaccharides, peptide, sterols, dietary fiber and lipids (Choi et al., 2013; Liu et al., 2010). These biologically active substances have received increasing attention and have been extensively used for preventing or attenuating human diseases, such as cancers (Hwang, Nam, Chang, Kim, & Noh, 2003), inflammatory diseases (Choi et al., 2013), hyperlipidemia (Chen, Ju, Li, & Yu, 2012a; Chen et al., 2012b), diabetes (Zong, Cao, & Wang, 2012), atherosclerosis (Kim et al., 2006) and liver damage (Chen et al., 2012a,b). However, there is no published information on the presence of functional substances in the *P. eryngii* residue generated during its production process (Fig. 1). Additionally, the information on the biological activities of the isolated polysaccharides generated by this study is of great significance in the development of functional foods with anticancer activity or in cancer therapy.

Polysaccharides extracted from *P. eryngii* have been demonstrated to have multiple bioactivities, such as enhancing immunity (Kang et al., 2004), antitumor (Kim et al., 2004), antihyperlipidemia (Chen et al., 2013), antioxidant (Kim et al., 2004), hepatoprotective (Chen et al., 2012a,b) and hypolipidemic (Chen et al., 2012a,b) activities. Previous studies have demonstrated that the antitumor activity of polysaccharides extracted from *P. eryngii* may be related to the activation of the immune response in tumor-bearing mice (Yang et al., 2013) and inhibiting the nuclear factor of activated T-cells (NF-AT)-, nuclear factor κB (NF-κB)- to suppress the level of IL-4 and allergy-related signaling proteins (Han et al., 2011). According to the previous studies, the biological activities of polysaccharides are closely associated with their structural features such as the molecular weights, chemical components, glycosidic

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Fig. 1. (A) Ripe *Pleurotus eryngii* in culture medium. (B) Fresh *Pleurotus eryngii* in the market. (C) *Pleurotus eryngii* residue with culture medium. (D) *Pleurotus eryngii* residue with no culture medium.

bonds, monosaccharide composition, polymerization, degrees of branching and 3-D conformation (Fan, Ding, Ai, & Deng, 2012a; Fan, Li, Deng, & Ai, 2012b; Zhang, Liu, Xiao, Zhang, & Sun, 2014). Several researchers have already confirmed that the acidic polysaccharide from *Lepista aordida* possessed more potent antiproliferative effect on human laryngocarcinoma HepG-2 cells than neutral polysaccharide (Miao et al., 2013). Many polysaccharides, especially the β-glucans isolated from various species of mushrooms have been intensively investigated in the medical fields for decades due to their immunomodulatory activities (Wang et al., 2013). However, limited correlation analysis is available between the structure and activity of polysaccharides.

In our previous study, two purified polysaccharide fractions from *Flammulina velutipes* were obtained and their antiproliferation activities *in vitro* have been investigated (Yang et al., 2012). In the present study, the polysaccharide fraction from the *P. eryngii* residue was purified into three main fractions, namely, PEPE-1, PEPE-2 and PEPE-3, by DEAE-52 ion-exchange and Sephadex G-100 column chromatographies. The structural features of these polysaccharides have also been preliminarily investigated. In addition, the tumor cells growth inhibitory effects and cytotoxicity of PEPE-1, PEPE-2 and PEPE-3 on HepG-2 human laryngocarcinoma cells were evaluated *in vitro* using MTT and LDH assays. Our results demonstrated that the three fractions were homogeneous polysaccharides mainly composed of glucose. Moreover, PEPE-3 had the highest molecular mass and was the most active fraction in inhibiting the growth of HepG-2 cells.

2. Experimental

2.1. Materials and chemicals

Fresh *P. eryngii* residue was purchased from a local market (Nanjing, China). Human liver cancer cell line (HepG-2) was obtained from Cell Bank of Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). DEAE-cellulose 52 and Sephadex G-100 resins were purchased from Whatman Co. (Maidstone, Kent, UK) and Pharmacia Co. (Sweden), respectively, while T-series dextran standards (T-500, T-200, T-100, T-50, and T-10) were purchased from Amersham Pharmacia (Uppsala, Sweden). The LDH assay kits were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). The monosaccharide standards (mannose, rhamnose, ribose, glucosamine, galacturonic acid, glucuronic acid, glucose, galactose, galactosamine, fucose, arabinose and xylose), dimethylsulfoxide (DMSO), bovine serum albumin (BSA), trifluoroaceric acid (TFA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco's minimal essential medium (DMEM), fetal bovine serum (FBS), fluorouracil (5-FU), penicillin and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). All other reagents were of analytical grade.

2.2. Preparation of crude polysaccharide

Fresh *P. eryngii* residue was cut into pieces to separate the culture medium and the fruiting bodies. Then, the cleaned residue was dried (60 °C), powdered and sieved through a No. 300 mesh. The powder was firstly refluxed with 85% EtOH at room temperature for 12 h to remove lipophilic compounds. After the mixture was centrifuged at 4500 × *g* for 15 min, the precipitate was extracted with distilled water under an optimized condition (ratio of water to material of 25 mL/g, leaching temperature of 70 °C, leaching time of 140 min). The whole extract solution was filtered and centrifuged at 4500 × *g* for 10 min, after the supernatant was deproteinized with Sevag reagent (chloroform: butanol, 4:1), 4-fold volume anhydrous ethanol was added to precipitate the crude polysaccharide for 12 h at 4 °C. Following centrifugation at 4500 × *g* for 15 min, the precipitate was washed successively with anhydrous ethanol and acetone, dialyzed against deionized water and lyophilized as crude *P. eryngii* residue polysaccharide.

2.3. Isolation and purification of PEPE

The crude polysaccharide (200 mg) was dissolved in 400 mL distilled water, after it was micro-filtrated through 0.45 μm membrane, the permeation solution was ultra-filtrated successively by ultra-filtration membranes with molecular weight cut off of 100 kDa (Millipore Co., Ltd.) under an optimized operation condition. Then, the retentate solution was subjected to a DEAE-52

cellulose column (2.6 cm × 30 cm) purification with a stepwise elution of 0–0.5 M NaCl solubilized in deionized water at a flow rate of 4 mL/min. Different fractions (10 mL/tube) namely P-1, P-2 and P-3 were collected according to the total carbohydrate content quantified by phenol–sulfuric acid method using an automatic fraction collector (Zhang, 1999). After dialysis, concentrated and lyophilized, P-1 and P-2 were further purified with a Sephadex G-100 column (2.6 cm × 60 cm) eluted with distilled water at a flow rate of 0.2 mL/min to yield three main final fractions (10 mL/tube), (PEPE-1, PEPE-2 and PEPE-3).

2.4. Homogeneity and molecular weight determination

The homogeneity and molecular weights of PEPE-1, PEPE-2 and PEPE-3 were determined by high-performance size-exclusion chromatography (HPSEC) on an Agilent 1200 system equipped with a TSK-gel G4000 PW_{XL} column (300 mm × 7.8 mm) and detected by an evaporative light scattering detector (ELSD) (Yang et al., 2012). The column and ELSD temperature were maintained at 30 °C. 20 μL sample solution (1 mg/mL) was injected into the detector and eluted with distilled water at a flow rate of 0.6 mL/min. Homogeneity and molecular weight analysis was estimated by the standard curve which was obtained with T-series dextran standards (T-500, T-200, T-100, T-50 and T-10).

2.5. Monosaccharide composition analysis

The monosaccharide composition analysis of PEPE-1, PEPE-2 and PEPE-3 was achieved by glyc-nitrile derivatization in gas chromatography (GC) analysis as described previously with slight modifications (Wang et al., 2012). Briefly, 5 mg of PEPE-1, PEPE-2 or PEPE-3 was hydrolyzed with 4 mL of trifluoroacetic acid (TFA, 2 mol/L) at 100 °C for 2 h. After the TFA/methanol solution in the hydrolyzates was removed by vacuum evaporation at 50 °C, 10 mg of hydroxylamine hydrochloride, 2 mg of inositol (as internal reference) and 0.6 mL of pyridine were reacted with the hydrolyzates at 90 °C for 30 min. Then, 1.0 mL of acetic anhydride was added into the mixture and incubated for another 30 min at 90 °C. All standard sugars (mannose, rhamnose, ribose, glucosamine, galacturonic acid, glucuronic acid, glucose, galactose, galactosamine, fucose, arabinose and xylose) were converted to their acetylated derivatives according to the abovementioned method. The acetylated derivatives of PEPE-1, PEPE-2 and PEPE-3 were analyzed by gas chromatography (GC) using an Agilent 6890 N instrument equipped with an HP-5 fused silica capillary column (30 m × 0.32 mm × 0.25 μm) and a flame-ionization detector (FID) according to the following temperature program: the oven temperature was initially set at 120 °C and maintained for 3 min, then increased to 210 °C at the rate of 3 °C/min and held at 210 °C for 4 min. The heater temperatures of the injector and detector were 250 °C and 280 °C, respectively. Nitrogen was used as the carrier gas. The area normalization method was used for the calculating of the molar proportions of monosaccharides in the polysaccharides.

2.6. Determination of sulfate and uronic acid contents

The content of sulfate groups in the polysaccharides was measured by the barium chloride–gelatin method using gelatin solution for the elimination of interference with some appropriate modifications (Dodgson & Price, 1962). Briefly, the polysaccharides mentioned above were hydrolyzed with 1 mol/L hydrochloric acid and then dried through a rotary evaporator. The dried residue was dissolved in 1 mL of water, reacted with 8% of trichloroacetic acid, 5% of barium chloride–gelatin solution and absorbances were measured at 360 nm after incubation for 20 min at room temperature. A standard curve was generated from the series of concentrations of potassium sulfate solution (0, 20, 40, 60, 80 and 100 μg/mL) and used to obtain a standard curve.

The determination of uronic acid content in the polysaccharides was achieved by sulfuric acid—carbazole method with some modifications (Karamanos, Hjerpe, Tsegenidis, Engfeldt, & Antonopoulos, 1988). Briefly, 0.2 mL polysaccharides solution (1 mg/mL) was mixed with 5 mL of sodium tetraborate sulfuric acid

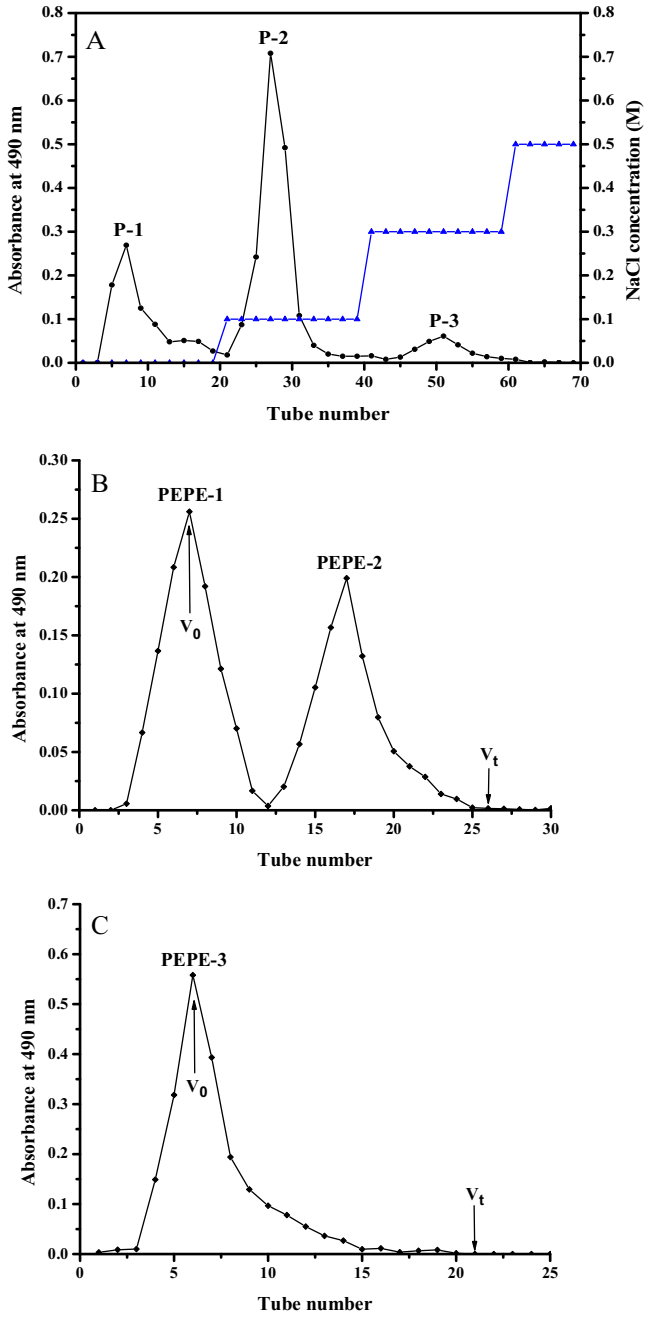

Fig. 2. (A) The elution profile of the crude polysaccharides isolated from *P. eryngii* residue on a DEAE cellulose-52 column eluted with distilled water and stepwise gradient of NaCl aqueous solutions (0, 0.1, 0.3 and 0.5 M) at a flow rate of 4.0 mL/min. (B) The elution profile of P-1 isolated from *P. eryngii* residue on Sephadex G-100 gel chromatography column (60 × 2.6 cm) eluted with distilled water at a flow rate of 0.2 mL/min (10 mL/tube), the PEPE-1 peak corresponds to the void volume (V_0), 70 mL; the tube No. 26 corresponds to the total column volume (V_t), 260 mL. (C) The elution profile of P-2 isolated from *P. eryngii* residue on Sephadex G-100 gel chromatography column (60 × 2.6 cm) eluted with distilled water at a flow rate of 0.2 mL/min (10 mL/tube), the PEPE-3 peak corresponds to the void volume (V_0), 60 mL; the tube No. 21 corresponds to the total column volume (V_t), 210 mL.

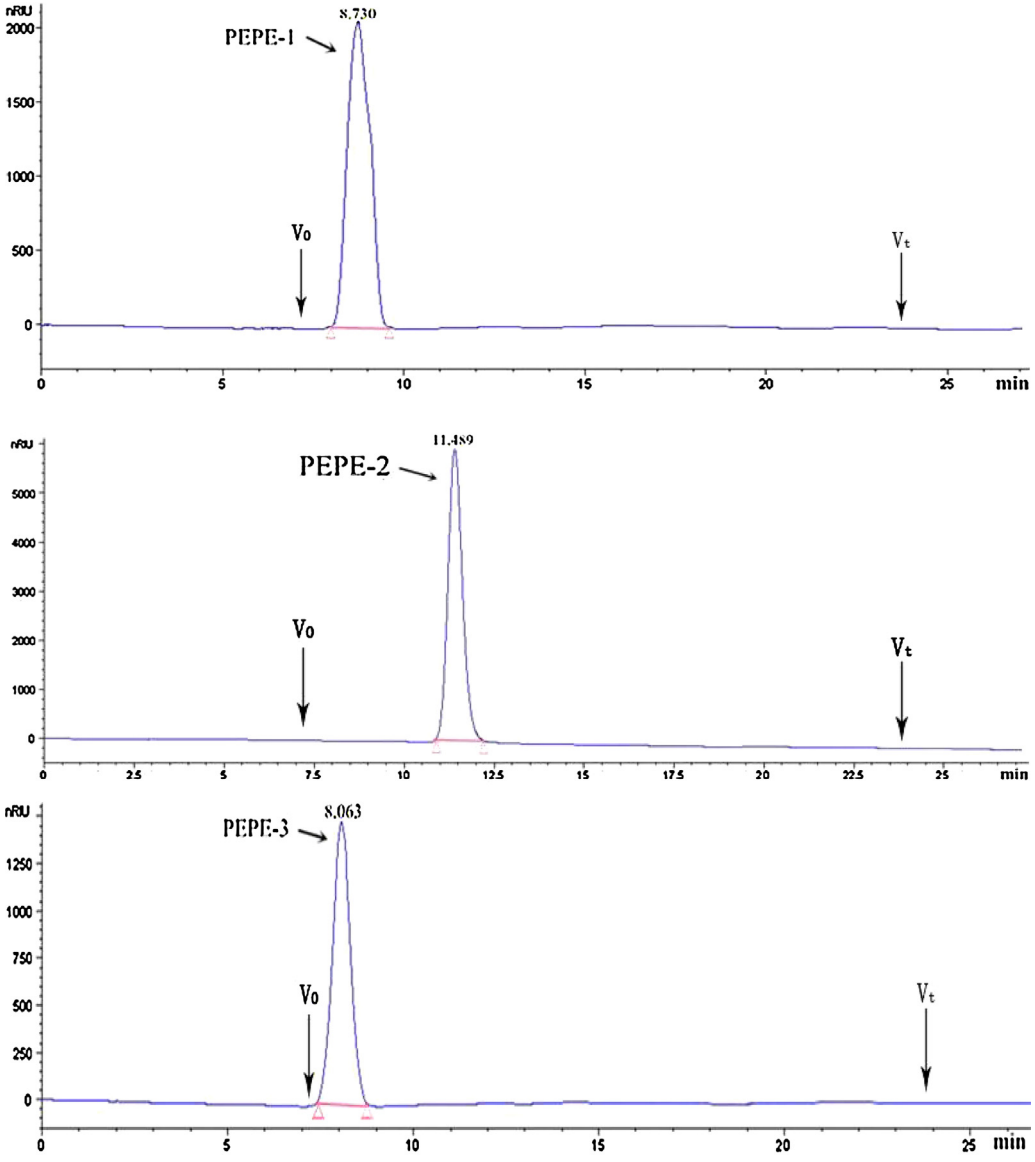

Fig. 3. High-performance size-exclusion chromatogram (HPSEC) of PEPE-1, PEPE-2 and PEPE-3 (V_0, 4.32 mL; V_t, 14.32 mL).

solution (9.54 mg/mL) and incubated in a boiling water bath for 10 min. Then, 0.2 mL of carbazole ethanol solution (1.25 mg/mL) was added into the solution for another reaction for 10 min. The absorbance of the solution was determined at 530 nm at room temperature while D-glucuronic acid served as the reference.

2.7. Ultraviolet (UV) and Fourier-transform infrared spectroscopy (FT-IR) assay

Ultraviolet and infrared spectra were performed for the determination of protein and nucleic acid contents in PEPE-1, PEPE-2 and PEPE-3. The ultraviolet spectra of the polysaccharides solutions were determined with an ultraviolet spectrophotometer (Shanghai Precision and Scientific Instrument Co. Ltd., Shanghai, China) in the range of 200–400 nm.

FT-IR from 500 to 4000 cm^{-1} was performed for the determination of the functional groups present in PEPE-1, PEPE-2 and PEPE-3 on a Nicolet Fourier transform infrared spectrometer (NICOLET NEXUS470, Thermo Nicolet Co., WI, USA).

2.8. Cell viability assay

The antitumor activity of PEPE-1, PEPE-2 and PEPE-3 on HepG-2 cells was evaluated using MTT assay (Miao et al., 2013). The cells

Table 1
Preliminary characteristics of PEPE-1, PEPE-2 and PEPE-3.

Sample	Molecular weight (kDa)	Uronic acid (%)	Sulfate (%)	Monosaccharides composition (%)[a]						
				Man	GlcN	Rham	Glc	Gal	Xyl	Ara
PEPE-1	208	1.05	1.65	8.01	–[b]	–[b]	74.82	11.15	1.24	–
PEPE-2	12	2.04	0.96	5.23	–	–[b]	86.74	5.12	–[b]	–
PEPE-3	413	2.16	1.13	4.08	–[b]	–[b]	90.93	2.89	–[b]	–

[a] Individual components were identified and quantified based on elution of known standards and data are presented as mol% for each sugar.
[b] Not detected.

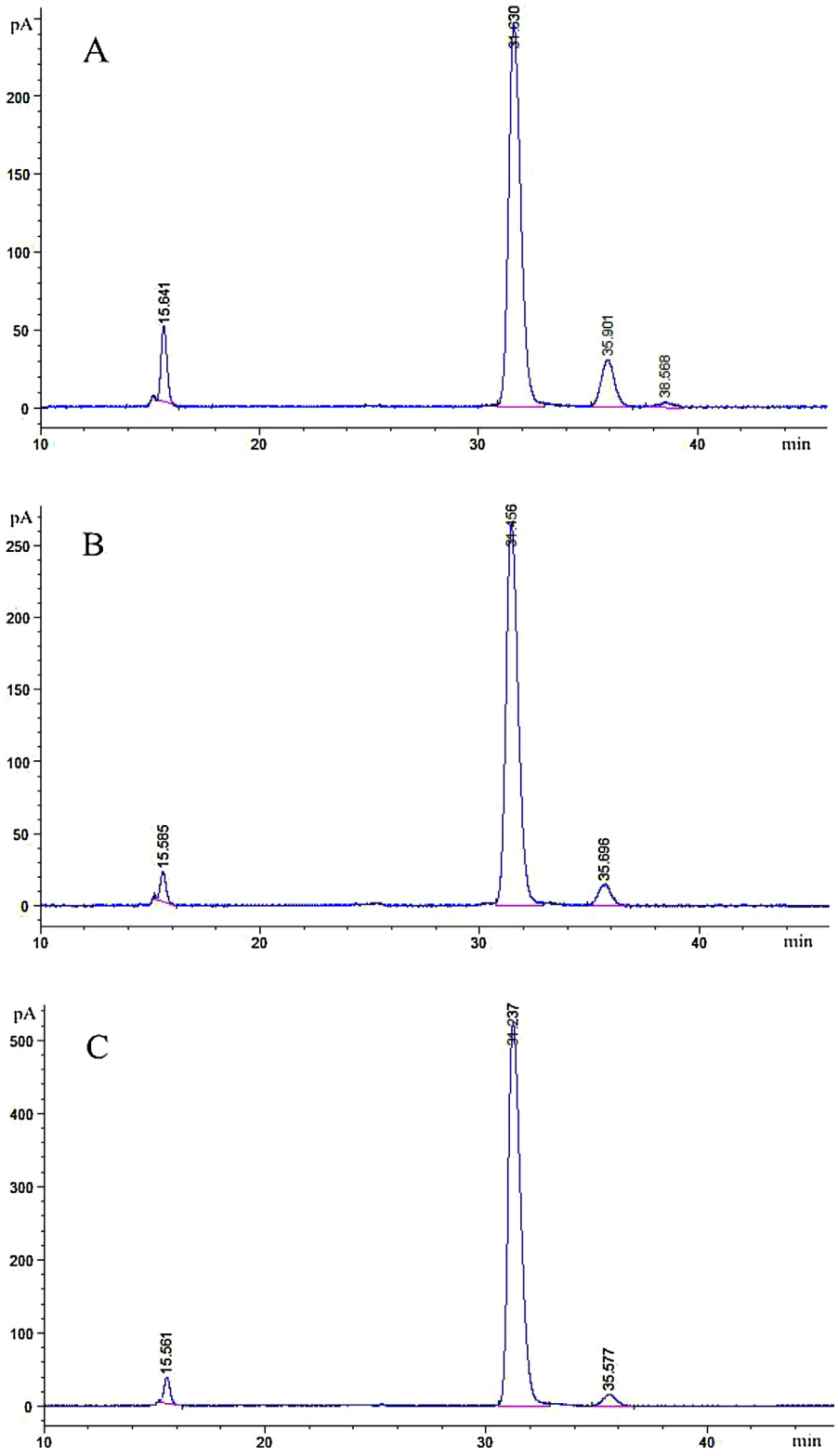

Fig. 4. (A) GC chromatograms of monosaccharides composition of PEPE-1, (B) PEPE-2 and (C) PEPE-3 with a HP-5 fused silica capillary column. Numbers above peaks indicated elution time.

were incubated in DMEM supplemented with 10% FBS, 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37 °C in a humidified incubator at 5% CO_2. Briefly, 100 μL of the cells were incubated in a 96-well plate at a concentration of 2×10^5 cells/mL. After 24 h of cultivation, various concentrations of PEPE-1, PEPE-2 and PEPE-3 (0, 10, 50, 100, 200 and 400 μg/mL) were added slowly into the 96-well plate and cultured for 16 h, 32 h and 48 h, respectively. 5-FU (50 μg/mL) served as the positive control. At the end of each treatment, 10 μL of MTT (5 mg/mL) was added and the tumor cells were further incubated for 4 h for the formation of the formazan crystals. A volume of 100 μL DMSO was added to each well to dissolve the formazan crystals after the medium was removed.

Subsequently, absorbance was measured at 570 nm with a microplate reader (Thermo multiskan Mk3, Thermo Fisher Scientific Inc., USA). The cell viability was expressed as

$$\text{Cell viability (\% control)} = \left[\frac{(A_s - A_b)}{(A_c - A_b)}\right] \times 100$$

where A_c and A_b were the absorbance of the system without the addition of polysaccharides or 5-FU and cells, respectively. And A_s was the absorbance of the system only with polysaccharides or 5-FU.

2.9. Cytotoxicity assay

Cytotoxicity of the polysaccharides was detected by measuring the release of LDH into the culture medium as an indicator of cell membrane injury (Chidambara Murthy, Jayaprakasha, Kumar, Rathore, & Patil, 2011) using a commercial LDH assay kit (Jiancheng BioEngineering, Nanjing, China) according to manufacturer's instructions. Briefly, at the end of the incubation period, 20 μL supernatant of the culture medium from different treatments was used to assess LDH leakage into the media. Subsequently, absorbance was measured at 450 nm with a microplate reader (Thermo multiskan Mk3, Thermo Fisher Scientific Inc., USA). The LDH release ratio (% control) was expressed as;

$$\text{LDH release ratio (\% control)} = \left[\frac{(A_s - A_b)}{(A_c - A_b)}\right] \times 100$$

where A_c and A_b were the absorbance of the system without the addition of polysaccharides or 5-FU and cells, respectively. And A_s was the absorbance of the system only with polysaccharides or 5-FU.

2.10. Statistical analysis

All experiments were carried out in triplicate and data were expressed as mean ± standard deviation. Statistical analysis was performed with one-way analysis of variance (ANOVA). Values of $P < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Isolation and purification of PEPE

The crude polysaccharide from hot water extraction was firstly loaded onto DEAE-52 cellulose anion-exchange chromatography column for the first-step purification based on the difference and quantity of ionic groups present (Lin et al., 2012). Three main fractions named P-1, P-2 and P-3 were collected with stepwise concentration (0, 0.1, 0.3 and 0.5 M) of sodium chloride solutions (Fig. 2A). P-1, eluted with deionized water could be a kind of neutral polysaccharide, while P-2 and P-3 eluted with 0.1 and 0.3 M NaCl solution, respectively, were acidic polysaccharides (Fan et al., 2012a,b). Because of the low yield of P-3, only P-1 and P-2 were subjected to a Sephadex G-100 gel filtration column for further purification according to the molecule distribution. Subsequently, through concentration, dialysis and lyophilization, PEPE-1, PEPE-2 and PEPE-3 were obtained and the elution profiles of the second purification were shown in Fig. 2B and C.

3.2. Molecular weight, monosaccharides composition, sulfate and uronic acid contents of PEPE-1, PEPE-2 and PEPE-3

Analysis with HPSEC revealed that both PEPE-1 and PEPE-2 displayed a single symmetrical peak, indicating that they were homogenous molecular weight polysaccharides as shown in Fig. 3 (Wang et al., 2013). The molecular weights of PEPE-1, PEPE-2 and PEPE-3 were 208, 12 and 413 kDa, respectively, in reference to the molecular weight-retention time equation developed by the calibration curve; $y = -0.4458x + 9.2113$, where y was the logarithm of the average molecular weight of the sample and x was the sample's retention time (Table 1).

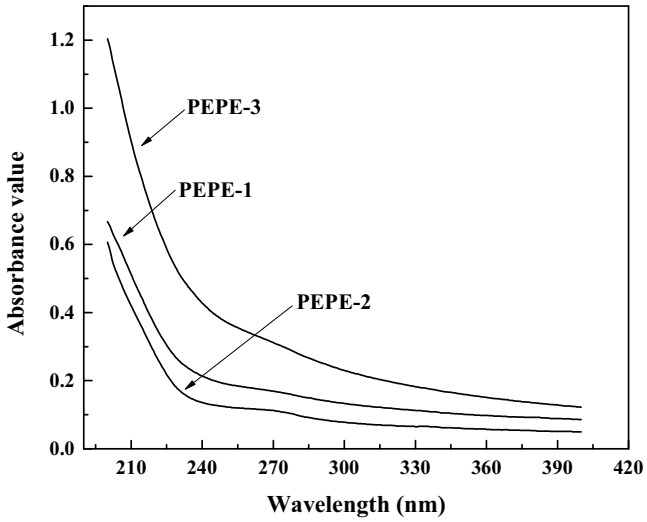

Fig. 5. UV spectra of PEPE-1, PEPE-2 and PEPE-3 in the range of 200–400 nm.

The composition and the molecular ratio of monosaccharides present in PEPE-1, PEPE-2 and PEPE-3 were shown in Fig. 4 and Table 1, respectively. PEPE-1 consisted of mannose, glucose, galactose and xylose in an approximate molar ratio of 8.01: 74.82: 11.14: 1.24, respectively; PEPE-2 was composed of mannose, glucose and galactose, in the molar ratio was 5.23: 86.74: 5.12, respectively, while PEPE-3 was composed of mannose, glucose and galactose with the molar ratio of 4.08: 90.93: 2.89, respectively. We can speculate that all the three polysaccharides were heteropolysaccharides with a glucan as backbone chain from the composition and molar ratio. Sulfate contents of PEPE-1, PEPE-2 and PEPE-3 were 1.65, 0.96 and 1.13%, respectively, and uronic acid contents of PEPE-1, PEPE-2 and PEPE-3 were 1.05, 2.04 and 2.16%, respectively (Table 1).

3.3. UV and FT-IR assay

The UV spectra of PEPE-1, PEPE-2 and PEPE-3 showed no absorptions at 260 nm and 280 nm, indicating the absence of nucleic acid and protein (Fig. 5). The FT-IR spectra of the three purified fractions ranging from 500 to 4000 cm^{-1} were showed in Fig. 6. The characteristic intense broad band around 3400 cm^{-1} was due to the hydroxyl groups (—OH) which was indicative of the strong inter- and intra-molecular interactions of the polysaccharide chains (Barker, Bourne, Stacey, & Whiffen, 1954). The weak absorption bands at about 2930 cm^{-1} were attributed to C—H stretching vibrations of the three polysaccharides (Yang et al., 2006). The peaks at around 2360 and 2340 cm^{-1} were the characteristic absorptions of aliphatic C—H bonds and C≡N groups, respectively (Gan, Ma, Jiang, Xu, & Zeng, 2011). The broad absorption bands with strong intensities around 1420 and 1374 cm^{-1} of PEPE-1 and PEPE-3 could be due to deforming vibrations of the C—H bond (Chen, Xie, Nie, Li, & Wang, 2008). The signal 1250 cm^{-1} was assigned to the stretching vibration of C=O groups (Rochas, Lahaye, & Yaphe, 1986). Two strong absorption peaks of PEPE-1, PEPE-2 and PEPE-3 within the range of 1100–1010 cm^{-1} indicated the possible presence of furanose ring in polysaccharides. The diagnostic absorption peak at about 902 cm^{-1} suggested that the glycosyl residues of PEPE-3 were mainly β-type glycosidic linkages (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000).

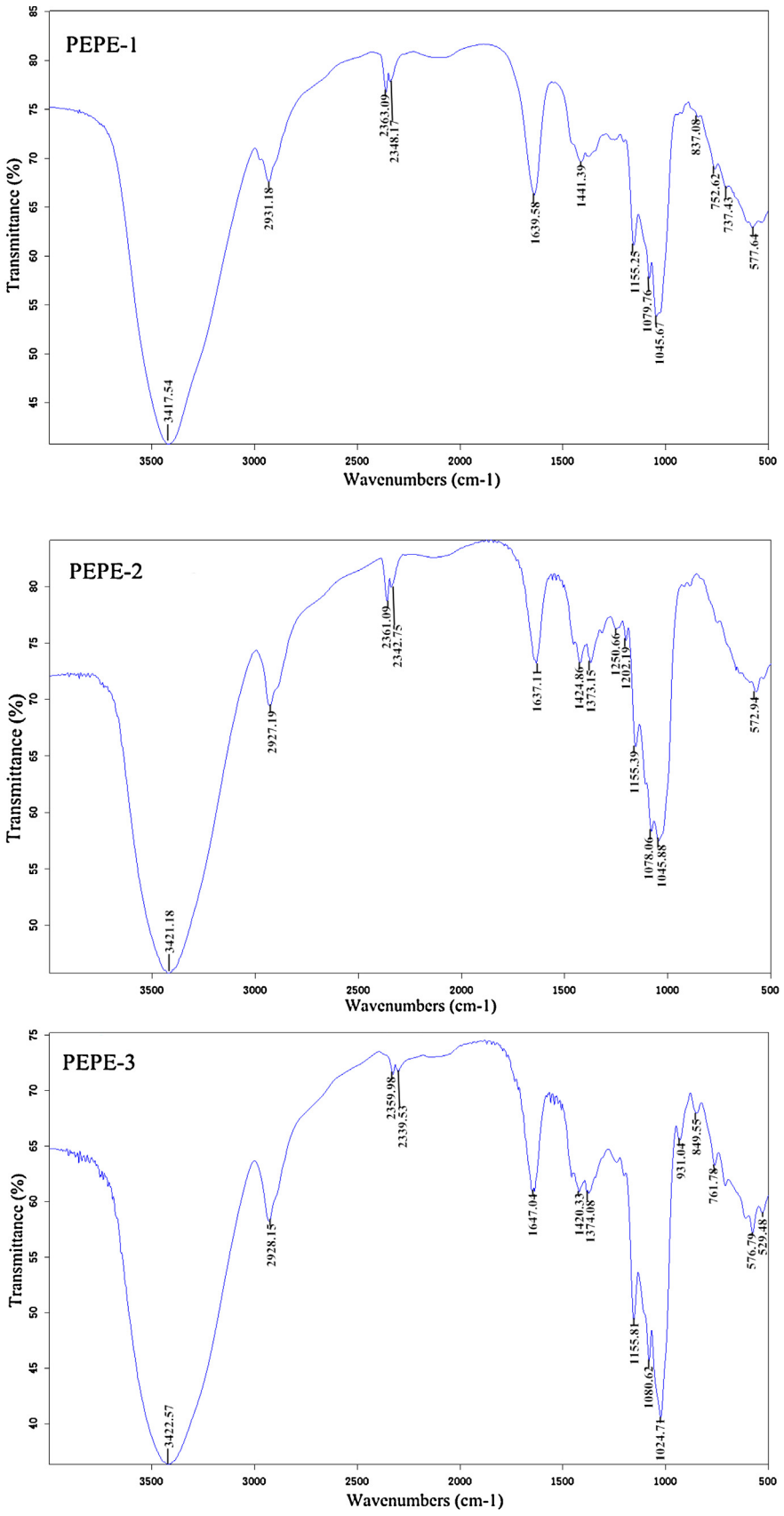

Fig. 6. FT-IR spectra of PEPE-1, PEPE-2 and PEPE-3 in the range of 500–4000 cm^{-1}.

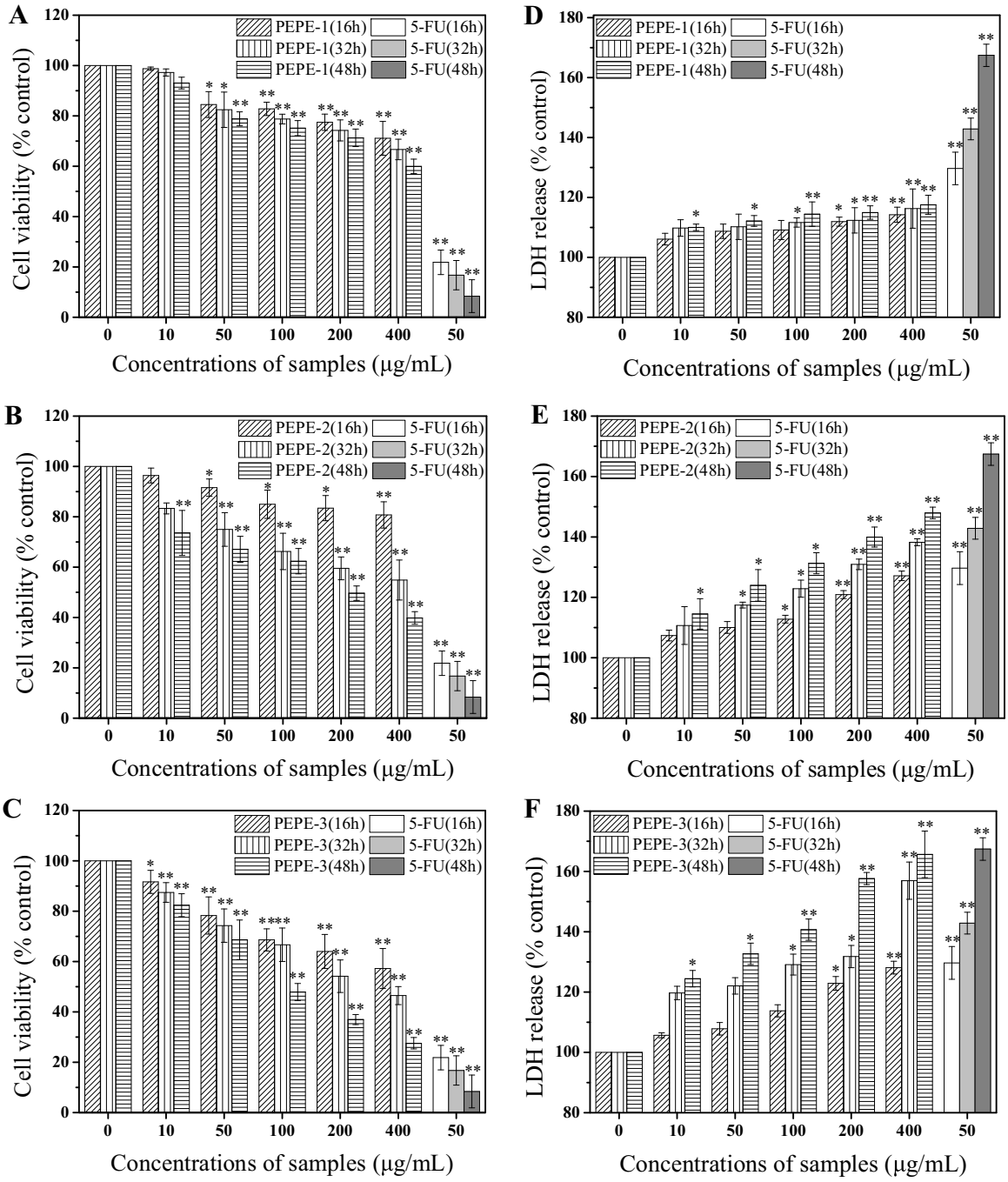

Fig. 7. ((A)–(C)) Effects of different polysaccharides from *P. eryngii* residue on the proliferation of HepG-2 cells as determined by the MTT assay; ((D)–(F)) cytotoxic effects of different polysaccharides from *P. eryngii* residue on HepG-2 cells as determined by LDH assay. The values are representative of three independent experiments; data are presented as mean ± S.D. (*) $P < 0.05$ and (**) $P < 0.01$ indicate statistically significant differences *versus* blank control groups.

3.4. Cell viability assay

The *in vitro* antitumor activity of PEPE-1, PEPE-2 and PEPE-3 were investigated at various concentrations (0, 10, 50, 100, 200 and 400 μg/mL) and incubation periods (16 h, 32 h and 48 h) against the growth of HepG-2 cells. As shown in Fig. 7A–C, PEPE-2 and PEPE-3 were demonstrated to significantly inhibit the proliferation of HepG-2 cells dose- and time-dependently. However, compared to PEPE-2 and PEPE-3, PEPE-1 had a lower inhibitory effect on the proliferation of HepG2 cells. At 400 μg/mL, PEPE-3 exhibited the highest growth-inhibitory effects against HepG-2 cells with a cell viability of 27.56 ± 2.28%, which was significantly ($P < 0.05$) higher compared with PEPE-2 at 400 μg/mL (39.82 ± 2.52%). PEPE-1 had moderate antitumor activity against HepG-2 cells at 400 μg/mL (59.96 ± 2.88%). The inhibitory effects of different polysaccharides on HepG-2 cells increased in the order of PEPE-3 > PEPE-2 > PEPE-1, the same as their uronic acid content. The results indicated that two of the polysaccharides (PEPE-2 and PEPE-3) extracted from the *P. eryngii* residue exhibited high specific antitumor activity toward HepG-2 cells.

3.5. Cytotoxicity assay

LDH assay was used to further confirm the proliferation inhibitory effects of PEPE-1, PEPE-2 and PEPE-3 on HepG-2 cells by evaluating the cytotoxicity of the three polysaccharides

(Wang et al., 2014). As shown in Fig. 7D–F, LDH leakage of HepG-2 cells was significantly increased in a dose- and time-dependent manner in the presence of the three polysaccharides in comparison to blank control ($P < 0.05$). Nevertheless, the increase of LDH release triggered by PEPE-1 treatment at 400 μg/mL after 48 h was only 117.56 ± 3.18% compared to the untreated cells, which was far less than that of PEPE-2 (147.95 ± 1.92%) and PEPE-3 (165.66 ± 7.72%). Interestingly, the cytotoxic strength of the three polysaccharides was the same as their uronic acid contents (PEPE-3 > PEPE-2 > PEPE-1). The results above indicated that PEPE-2 and PEPE-3 was endowed with higher cytotoxic effects against the HepG-2 cells, which was consistent with the MTT assay results.

4. Conclusions

In the present study, crude polysaccharide was obtained from *P. eryngii* residue using hot water. After purification by DEAE-52 and Sephadex G-100, three fractions namely PEPE-1, PEPE-2 and PEPE-3 with molecular weights of 208, 12 and 413 kDa, respectively, were obtained. Monosaccharides analysis revealed that they were all mainly composed of glucose at the ratios of 74.82, 86.74 and 90.93%, respectively. UV and FT-IR spectra showed that there was no nucleic acid and protein in these three fractions and PEPE-3 had mainly β-type glycosidic linkages. The *in vitro* antitumor activities of these three fractions have been revealed *via* MTT and LDH assays. The results showed that PEPE-2 and PEPE-3 exhibited significant antitumor activity against HepG-2 cells whereas PEPE-1 had moderate activity, which could be related to their structural characteristics. These characteristics include molecular weight, monosaccharides composition, sulfate and uronic acid contents. These results suggested that the polysaccharides (PEPE-1, PEPE-2 and PEPE-3) obtained from the *P. eryngii* residue might be suitable for use as functional foods and potential therapeutic agents for human cancer disease. However, more studies are required to explore their complete structural characteristics, structure–activity relationship and the mechanism of their antitumor activity.

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