Original Article

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Structural characterization and biological activities of a novel polysaccharide from *Phyllanthus emblica*

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Summary **A novel water-soluble polysaccharide named PEPW80-1, with a molecular mass of 4.7 kDa, was isolated from the pulp tissues of *Phyllanthus emblica*, and purified by sephadex G-100 column and sephacryl S-300 HR chromatography. The structural features of PEPW80-1 were investigated by a combination of acid hydrolysis, periodate oxidation-Smith degradation, methylation analysis, gas chromatography-mass spectrometry, scanning electron microscope, Fourier transform infrared spectroscopy, and nuclear magnetic resonance spectroscopy. The results showed that PEPW80-1 had a specific optical rotation of $[\alpha]_D^{25} = +113°$ (c = 0.5 mg/mL) and its backbone composed of (1,3)-linked-*β*-L-rhamnose and (1,3,6)-linkage-*β*-D-galactose, with two branch chains of (1,4)-linked-α-D-galactose and (1,6)-linked-*β*-D-galactose and terminated with 1-α-L-arabinose. The antioxidant assays showed that PEPW80-1 possess 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity and hydroxyl radical-scavenging activity, enhancing reductive power. The results of immunomodulatory assays *in vitro* showed that PEPW80-1 could promote the proliferation of mouse splenocytes. Those proposed that PEPW80-1 might be developed as a potential value-added product with the activities of immunomodulator and free-radical inhibitors.**

Keywords: *Phyllanthus emblica*, polysaccharide, structure characterization, antioxidant activities, immunomodulatory activity

1. Introduction

In recent years, polysaccharides extracted from plants, animals and microorganisms have attracted increasing attention due to their unique biological activities (*1*), such as antioxidant activity (*2*), hepatoprotective activity (*3*), antitumor activity (*4*), immunomodulatory activity (*5*) and interferon-inducing activity (*6*). Many studies have demonstrated that botanical polysaccharides have the potential to activate cells involved in innate immunity (*7*). Interestingly, the botanical polysaccharides have also been proven to possess high antioxidant activities (*8*). Literature also indicates that the herbal antioxidants concurrently exhibit significantly immunomodulatory activities (*9*). It is therefore of great interest to investigate immunomodulatory effects of herbal polysaccharides that exhibit antioxidant activity with low toxicity.

Oxidation is essential to many organisms for the production of energy to fuel biological processes. However, reactive oxygen species are often over-produced under pathological conditions, resulting in oxidative stress (*10*). There are many kinds of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxyl radical and singlet oxygen (*11*). Although ROS at physiological concentration may be required for normal cell function, excessive amount of ROS can damage cellular components such as lipids, proteins and DNA. Many diseases such as cancer, cardiovascular

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diseases, rheumatoid arthritis, and atherosclerosis are believed to be related to the production of excessive amounts of ROS in the body (*12*). The free radical theory of aging suggests that the damage produced by the interactions of such free radicals with cellular macromolecules results in cellular senescence and aging. Although almost all organisms possess antioxidant and repair systems to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely (*13*).

There is increasing evidence that many kinds of polysaccharides isolated from plants, fungi and bacteria are associated with immunostimulatory properties without significant side effects (*14*). The spleen combines the innate and adaptive immune system in a uniquely organized way, which has been the most important organ for antibacterial and antifungal immune reactivity. Therefore, the spleen has been an ideal tissue in immune research (*15*).

Phyllanthus emblica is extensively distributed in subtropical and tropical areas of China, India, Indonesia, and Malaysia and well known by consumers for its unique taste. The fruit of *P. emblica*, which has been used widely for thousands of years in Chinese and Indian traditional medicinal systems (*16*), was reported to have lipid-lowering (*17*) and hypoglycemic activities (*18*). It acts as not only an important constituent of many hepatoprotective formulations available (*19*), but also an antimicrobial (*20,21*), anticancer (*22,23*) and antiinflammatory agent (*24,25*), improving metal-induced clastogenic effects (*26*).

In the present study, we report for the first time the extraction, purification, structural characterization and ultrastructure of PEPW80-1 from the pulp tissues of *P. emblica* and evaluate its antioxidant and immunomodulatory activities *in vitro*.

2. Materials and Methods

2.1. *Materials and reagents*

Kunming mice (male, 8-12 weeks old, 18.0-20.0 g, License No. SCXK, 2011-0029) were purchased from Experimental Animal Center of Sun Yat-sen University, Guangzhou, Guangdong province, China. All mice were kept at the animal facilities under specific pathogen-free condition until used. Sterile food and water were supplied.

The fresh fruits of *P. emblica*, at the commercially mature stage, were purchased from Jieyang, Guangdong province, China. The material was identified by Professor R.M. Yu, College of Pharmacy, Jinan University, China. The fruit was dried at 55°C, and the pulp tissues were carefully removed from the seeds.

Diethylaminoethyl (DEAE) cellulose-52 was obtained from Whatman Ltd. (England). Sephadex G-100 and Sephacryl S-300 HR was purchased from Amersham Biosciences (Swenden). Ascorbic acid (vitamin C, Vc), H_2O_2, ferrous sulfate ($FeSO_4$), and sulfate acid (H_2SO_4) were obtained from Guangzhou Chemical Reagent Co., Guangzhou, Guangdong province, China. All other reagents were obtained from Sigma Chemical Co. (USA) and all reagents were of analytical grade. Dimethylsulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium- bromide (MTT), trypan, concanavalin A (Con A), penicillin G and streptomycin were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. *Preparation and isolation of polysaccharides*

Preparation of the crude polysaccharide (PEPW80) from *P. emblica* was performed by water extraction and ethanol precipitation (*27*). *P. emblica* powder was extracted with 95% ethanol and then with distilled water for 2 h at 80°C three times. The water extracts were combined, filtered, concentrated, and then precipitated by addition of four-fold-volume of 95% ethanol. The precipitate was collected by centrifugation and dissolved by distilled water. The solution was deproteinated by a combination of papain enzymolysis and Sevag method, then dialyzed against tap water and distilled water, each for 48 h and lyophilized to obtain PEPW80. The obtained polysaccharide was applied to DEAE-52 cellulose column (1.6 × 50 cm) equilibrated with distilled water. The polysaccharide was fractionated and eluted at 0.7 mL/min of distilled water and sodium chloride (NaCl) solutions (0-0.8 mol/L), respectively. The main fractions were combined according to the total carbohydrate content quantified by the phenol-sulfuric acid method. Then one of fractions PEPW80-b was further fractionated with size-exclusion chromatography on a sephadex G-100 column (1.6 × 50 cm) and eluted at the flow rate of 0.3 mL/min with deionized water. The fraction was collected, concentrated, dialyzed and lyophilized to obtain a purified polysaccharide (PEPW80-1). The PEPW80-1 was then stored in bottle desiccators at room temperature for further study.

2.3. *Measurement of molecular weight of PEPW80-1*

Molecular weight of PEPW80-1 was determined by gel permeation chromatography on a column of sephacryl S-300 HR (2.6 × 80 cm) with water as the eluant at a flow rate of 0.3 mL/min. The column was calibrated with glucose, standard T-series dextran and blue dextran, respectively. Elution volume of polysaccharide was plotted in the same graph, and then the molecular weight of PEPW80-1 was determined.

2.4. *Analysis of monosaccharide composition*

PEPW80-1 (5.0 mg) was hydrolyzed with 2.0 M trifluoroacetic acid (TFA, 10 mL) at 100°C in a reaction

tube for 6 h. Excess acid was removed by evaporation on water bath at 60°C and co-distilled with methanol after the hydrolysis was completed. The hydrolysate (1.0 mg) was dissolved in pure water (1.0 mL) and used for the ion chromatographic analysis by high performance anion exchange chromatography-pulsed amperometric detector (HPAEC-PAD) on the Dionex ICS-2500 system, eluted with a mixture of water and 200 mM sodium hydroxide (NaOH) in the volume ratio of 92:8 (*28*).

2.5. *Partial acid hydrolysis of PEPW80-1*

Fractions 1 and 2 were hydrolyzed with 2 M TFA, after excess TFA in the fraction was removed by co-distillation with methanol (1 mL × 3), and fraction 3 was tested by HPAEC-PAD, respectively (Figure 1).

2.6. *Periodate oxidation–Smith degradation*

The periodate oxidation analysis was performed as described in reference (*29*). The sample (25 mg) was dissolved in 0.015 M sodium periodate ($NaIO_4$) (25 mL) and the solution was kept at 4°C in dark. The A_{223} nm of the reaction solution was measured every 6 h with a spectrophotometer. After the oxidation reaction was completed (96 h), the excess $NaIO_4$ was decomposed with ethylene glycol (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 0.01 M NaOH. The reaction mixture was dialyzed against tap water and then distilled water, each for 48 h. After dialysis, the retentate was freeze-dried, hydrolyzed with 2 M TFA (1 mL) at 120°C for 2 h, reduced by sodium borohydride (NaBH4), 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).

2.7. *Fourier transform infrared spectrophotometer (FT-IR) analysis*

FT-IR spectrum of the sample was determined using a Fourier transform infrared spectrophotometer (Nexus 5DXC FT-IR, Thermo Nicolet, America). The sample was grounded with spectroscopic grade potassium bromide (KBr) powder and then pressed into a 1 mm pellet for FT-IR measurement in the frequency range of 4,000-400 cm^{-1} (*27*).

2.8. *NuclearMagneticResonance (NMR) analysis*

The freeze-dried polysaccharide was kept over phosphoric anhydride (P_2O_5) in vacuum for several days. The deuterium-exchanged polysaccharides (30 mg) were put in a 5-mm NMR tube and dissolved in 0.5 mL 99.96% deuterium oxide (D_2O). ^{13}C NMR spectrum was recorded with a Bruker AM 500 MHz spectrometer (Bruker, Rheinstetten, Germany), operating frequencies 100.61 MHz for ^{13}C NMR at 30°C. Chemical shift was

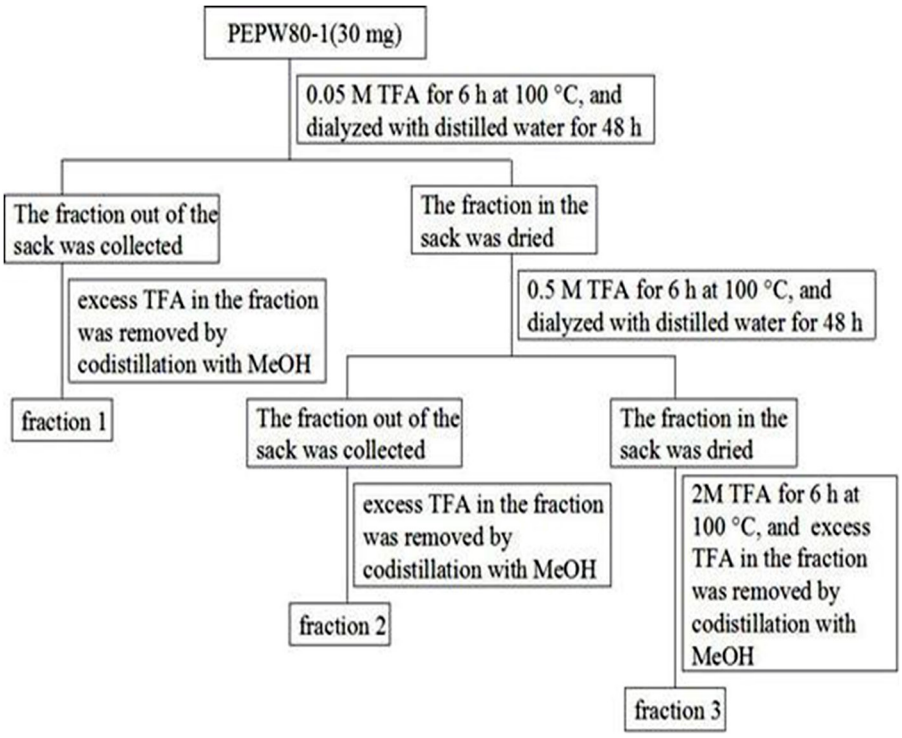

Figure 1. Procedure of partial acid hydrolysis of PEPW80-1.

expressed in ppm. Tetramethyl-silane was used as an internal standard.

2.9. *Scanning electron microscopy analysis*

Specimen was glued on specimen stubs using silver conducting tape and coated with gold–palladium using a sputter coater (BAL-TEC SCD 500, Liechtenstein). Scanning electron micrographs were obtained using an environmental scanning electron microscopy (SEM) (XL 30 ESEM, Philips, Holland) with the parameters HJ: 20 kV, resolution ratio: 3.4 nm, and spot: 4.

2.10. *Determination of antioxidant activities in vitro of PEPW80-1*

2.10.1. *DPPH radical-scavenging assay*

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method with slight modification (*28*). The solution of DPPH radical (0.1 mM) in methanol was prepared and 1.0 mL of this solution was added to 3.0 mL of the polysaccharide of different concentrations (0.1-3.2 mg/mL) in water. The mixture was shaken and incubated at 25°C for 30 min in the dark, and then the absorbance was measured at 517 nm against a blank (water instead of test sample and DPPH radical solution). Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. The percent scavenging activity was calculated by the following formula:

$$\text{Scavenging rate (\%)} = [1 - (A_1 - A_2)/A_0] \times 100$$

where A_0 is the absorbance of the control (water instead of test sample solution), A_1 is the absorbance of the sample. A_2 is the absorbance of the sample under identical conditions as A_1 and with water instead of DPPH radical solution.

2.10.2. *Hydroxyl radical-scavenging (HOSC) assay*

Hydroxyl radicals were generated using an innovative method (*30*). Sodium phosphate buffer (3 mL, 150 mM, pH 7.4), which contained 10 mM ferrous sulfate ($FeSO_4$), 2 mM sodium salicylate, 6 mM H_2O_2, and different concentrations (0.1-3.2 mg/mL) of polysaccharide, were incubated at 37°C for 1 h. The absorbance was detected at 510 nm, and Vc was used as a positive control. The percent scavenging activity of hydroxyl radicals was calculated as follows:

$$\text{Scavenging rate (\%)} = [(A_s - A_0)]/[(A - A_0)] \times 100$$

where A_s is the absorbance in the presence of the sample, A_0 is the absorbance of the control in the absence of the sample, and A is the absorbance without the sample and Fenton reaction system.

2.10.3. *Ferric-reducing antioxidant power*

Ferric-reducing antioxidant power (FRAP) potential of PEPW80-1 was determined according to the modified method by Nakajima (*31*). Different concentrations (0.1-3.2 mg/mL) of PEPW80-1 was mixed with 2.5 mL of 0.2 M phosphate buffered saline (PBS) (pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$). The mixture was incubated at 50°C for 20 min. Aliquots (2.5 mL) of 10% trichloroacetic acid were added to the mixture, which was then centrifuged for 10 min at 1,500× g. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, and the absorbance was measured at 700 nm using UV-vis spectrophotometer (UV-2450, Shmadzu, Japan).

2.11. *Measurement of immunomodulatory activity*

2.11.1. *Cells and animals*

Splenocytes were prepared from mice as described by Kim (*6*). The cells were freed of red blood cells by treatment with lysis buffer (0.15 M ammonium chloride (NH_4Cl), 0.01 M potassium bicarbonate ($KHCO_3$), and 0.1 mM sodium ethylene diamine tetracetate (Na_2EDTA), pH 7.4). To remove adherent cells such as macrophages, splenocytes were incubated for 2 h in Petri dishes at a concentration of 5×10^6 cells/mL. The suspended cell populations were collected and used as the splenocytes populations.

2.11.2. *Splenocytes proliferation*

The splenocytes proliferation was assessed by using MTT-based colorimetric assay as previously described (*32*). An aliquot of 100 µL of splenocytes was seeded into 96-well flat bottom microtitre plates, thereafter the polysaccharide (final concentration 25, 50, 100, 200 and 400 µg/mL) was added, giving a final volume of 200 µL. Con A was used for reference purposes at a final concentration of 10 µg/mL. After incubation at 37°C in a humid atmosphere with 5% carbon dioxide (CO_2) for 48 h, 50 µL of MTT solution (1 mg/mL) was added to each well and incubated for another 6 h. The plate was centrifuged on 1,000× g for 5 min and the supernate was discard, and then 150 µL DMSO was added per well. Absorbance at 570 nm was measured on a microplate reader (Multiskan Spectrum, Thermo Scientific Instruments).

2.12. *Statistical Analysis*

All experiments were repeated three times. Results are presented as the mean ± the standard error of the mean (SEM). Comparison of the data was performed using the single-factor analysis of variance (ANOVA) test. Significance was defined as a p value of < 0.05.

3. Results

3.1. *Fractionation of PEPW80-1*

Two fractions consecutively eluted by 0-0.8 mol/L NaCl through an anion-exchange column were respectively coded as PEPW80-a and PEPW80-b. The yield of PEPW80-a and PEPW80-b respectively accounted for 5.08% and 56.02% of the crude polysaccharide extract, while the ratio of their peak areas was 3.07:35.02, indicating that PEPW80-b was the major component. PEPW80-b was further purified on a Sephadex G-100 (GE Health Care Biosciences AB, Uppsala, Sweden) gel filtration column (600 × 16 mm), and then the collected fraction was checked by sephacryl S-300 HR column which showed a single, symmetric, and sharp peak, named PEPW80-1, indicating near-homogeneity, with purity of 98.4% (data not shown). Surface and clear shape of refined polysaccharides could be observed with SEM. SEM analysis of the membrane sample provides a qualitative indication of the nature of polysaccharides. As shown in the Figure 2, the amber-colored loose powder PEPW80-1 was smooth and regular. In addition, PEPW80-1 contained no protein and nucleic acid, as evidenced by the lack of absorbance near 260 and 280 nm.

3.2. *Structural elucidation of PEPW80-1*

PEPW80-1 was consisted of rhamnose, arabinose and galactose (3.02:1.00:4.23). Its initial structural features were analyzed by partial acid hydrolysis, methylation, FT-IR and ^{13}C NMR.

The average molecular weight of the PEPW80-1 was determined to be 4.7 kDa by gel permeation chromatography (GPC) technique on a Sephacryl S-300 HR column. Calibration was performed with dextran molecular weight standards. In the FT-IR spectrum of PEPW80-1, the strong band at 3,425.4 cm^{-1} was attributed to the hydroxyl stretching vibration of the polysaccharide, and that at 2,926.6 cm^{-1} was due to the C-H stretching vibration absorption. The absence of any bands at 1,735.0 cm^{-1} confirmed that there was no uronic acidic in this fraction. The band around 1,624.7 cm^{-1} was due to the bound water. The absorption band at 890.0 cm^{-1} indicated that PEPW80-1 contained a *β*-type glycosidic linkage in its structure and the characteristic bands at 1,000.0-1,100.0 cm^{-1} suggested the presence of pyranose form of the glucosyl residue.

The analysis results of fractions 1 and 2 showed that the branched structure of PEPW80-1 was composed of galactose and terminated with arabinose, and that of fraction 3 indicated that rhamnose, galactose could be the backbone of the structure of PEPW80-1. The periodate oxidized products were fully hydrolyzed and analyzed by GC after 2M TFA hydrolysis. The results showed that there were galactose, rhamnose in the oxidation products. The presence of rhamnose and galactose indicated that some residues of rhamnose and galactose were 1,3-, 1,2,3-, 1,2,4-, 1,3,4-, 1,3,6- or 1,2,3,4-linked, which cannot be oxidized. At the same time, a part of galactose were 1,4- and 1,6-linkage, which might be oxidized to produce erythritol and glycerol.

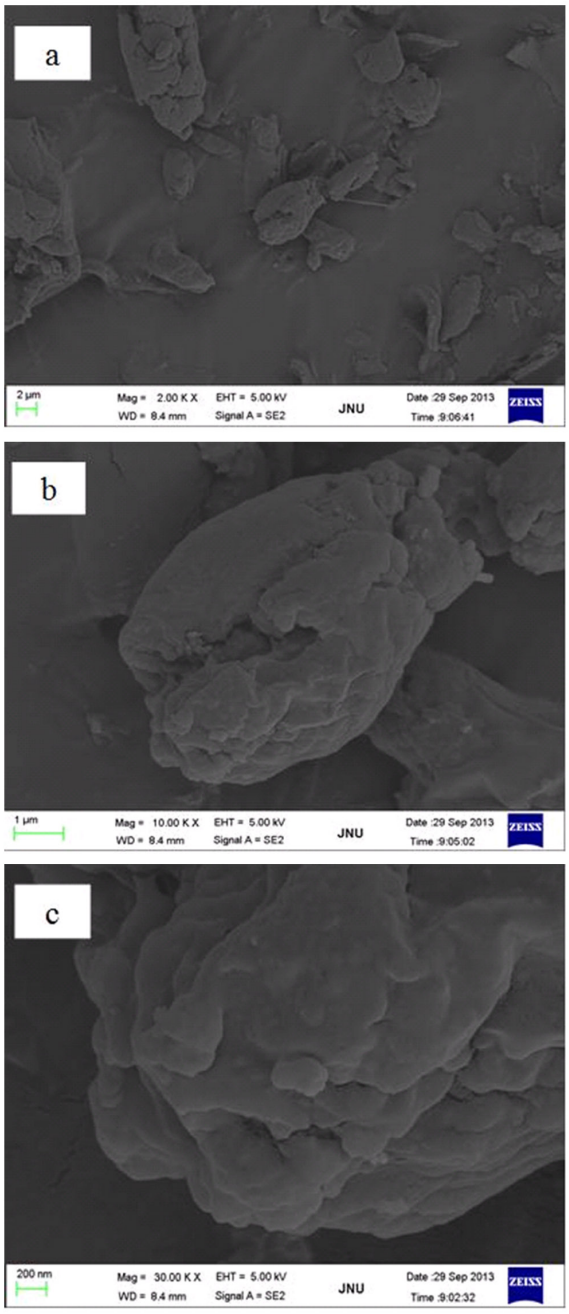

Figure 2. PEPW80-1 of imaged by SEM. a: 2.00 KX, **b:** 10.00 KX, **c:** 30.00 KX.

NMR spectroscopy has been the most powerful technique for the structure analysis of carbohydrates (*33*). The data (δ, ppm) of ^{13}C NMR of PEPW80-1 was shown in Figure 3, and five signals appeared in the anomeric region, suggesting the presence of five different linkage patterns. The data of ^{13}C NMR

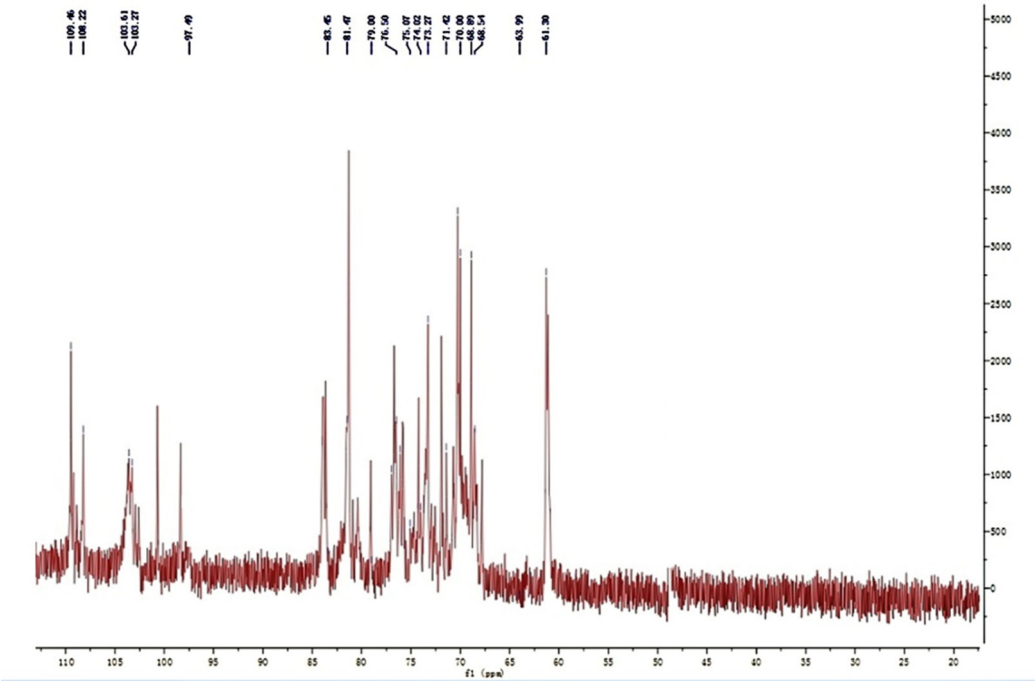

Figure 3. ^{13}C-NMR spectrum of PEPW80-1.

Table 1. Assignment of ^{13}C NMR chemical shifts of PEPW80-1

Sugar residue	Chemical shifts (ppm)						
	C1	C2	C3	C4	C5	C6	CH_3
α-L-Araf(1→	109.2	81.5	76.5	83.8	64.0		
→3)-*β*-L-Rhap(1→	108.3	78.9	83.8	76.7	74.1		16.4
→6)-*β*-D-Galp(1→	103.7	74.1	76.1	71.4	75.1	64.0	
→3,6)-*β*-D-Galp(1→	103.3	73.2	77.0	69.9	76.7	68.8	
→4)-α-D-Galp(1→	97.21	70.3	69.8	78.9	68.8	61.3	

identified the signals as 1,6-*β*-D-galactopyranose (δ 103.7), 1,3,6-*β*-D-galactopyranose (δ 103.3), 1,3-*β*-L-rhamnopyranose (δ 108.3) 1,4-α-D-galactopyranose (δ 97.2) and 1-α-L-arabino- furanose (δ 109.2) terminal residues. The result also proposed that the backbone was composed of 1,3,6-*β*-D-galactopyranose and 1,3-*β*-L-rhamnopyranose residues, with two branch chains of (1,4)-linked-α-D-galactose and (1,6)-linked-*β*-D-galactose and terminated with 1-α-L-arabinose (*34-35,28*). The assignment of the carbon atom signals was shown in Table 1.

According to above elucidation, PEPW80-1 could be proposed as a heteropolysaccharide, with a backbone of 1,3-*β*-L-rhamnose and 1,3,6-*β*-D-galactose, two branch chains of 1,6-*β*-D-galactose and 1,4-α-D-galactose based on the experimental results. The terminal residue was 1-α-L-arabinose. On the basis of above discussion, the structure of PEPW80-1 might be assigned as Figure 4.

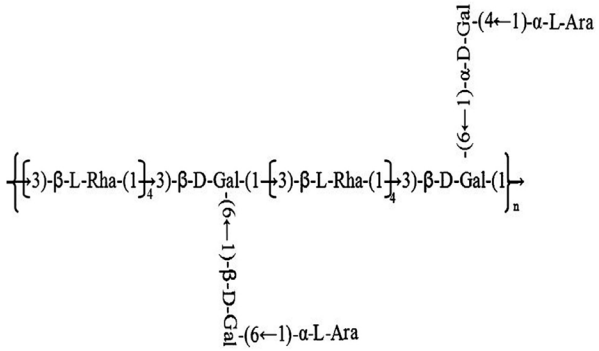

Figure 4. The proposed structure of PEPW80-1.

3.3. *Methylation analysis by GC-MS*

Methylation analysis by GC-MS was used to provide more structural information for PEPW80-1. The results shown as 2,4-Me_2-Rha, 2,3,6-Me_3-Gal, 4,6-Me_2-Gal, 2,3,4-Me_3-Gal, 2,3,4-Me_3-Ara in a molar ratio of 4.03:1.07:1.05:1.00:1.15 (Table 2). Therefore, it suggested that the repeating unit of PEPW80-1 consisted of 1,3-linked galactose, 1,4,6-linked galactose, 1,6-linked galactose, 1,3-linked rhamnose and 1-linked glucose.

Table 2. Glycosidic linkage composition of methylated PEPW80-1

Methylation sugar	Molar ratio	Mass fragments (m/z)	Linkage type
2,3,6-Me_3-Gal	1.07	43, 59, 72, 118, 233	1→4
2,4-Me_2-Gal	1.05	43,86, 117, 129, 189	1→3,6
2,3,4-Me_3-Gal	1.00	43, 71, 101, 129, 161, 189, 233	1→6
2,3,4-Me_3-Ara	1.15	43, 45, 71, 87, 101, 117, 129, 161	1→
2,4-Me_2-Rha	4.03	43, 59, 69, 75, 85, 99, 101, 117, 129	1→3

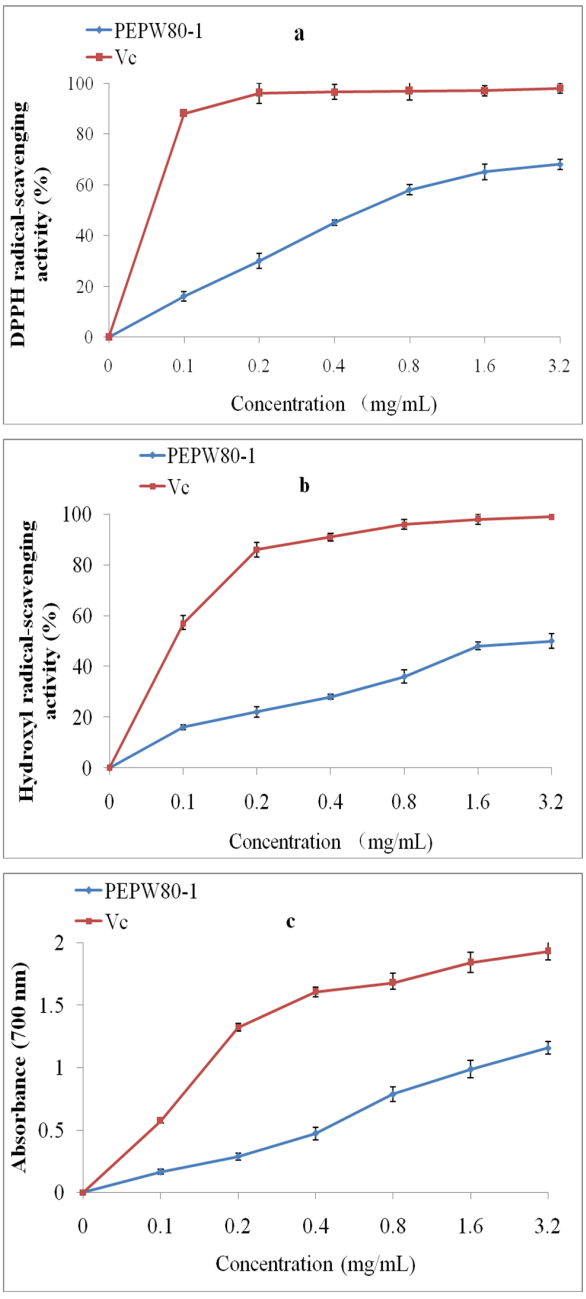

Figure 5. Antioxidant activities of PEPW80-1. a: DPPH radical-scavenging activity, **b:** Hydroxyl radical-scavenging activity, **c:** Ferric-reducing activity. Values are means ± SD of three separate experiments.

The molar ratio of these residues agreed overall with the monosaccharide composition of PEPW80-1 described above.

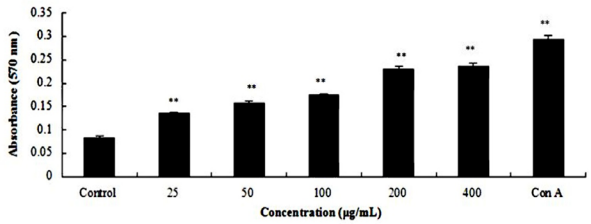

Figure 6. Effects of PEPW80-1 promoted splenocyte proliferation. Values are means ± SD of three separate determinations.

3.4. *Antioxidant activity of PEPW80-1*

3.4.1. *Scavenging activity to the DPPH radical*

As shown in Figure 5a, the results of scavenging activity to the DPPH radical indicated that PEPW80-1 showed obvious scavenging activity on DPPH radical in a concentration-dependent manner at relatively low concentration (0.0-0.8 mg/mL). The maximum value of PEPW80-1 reached 69.2% that of Vc. The IC_{50} values of PEPW80-1 was 0.5 mg/mL.

3.4.2. *Scavenging effects on hydroxyl radicals*

Removing hydroxyl radical is important for the protection of living systems. The results of hydroxyl radical scavenging (HOSC) activities of the PEPW80-1 and Vc were given in Figure 5b. The scavenging effect of PEPW80-1 was enhanced significantly with the increase of sample concentration ranging from 0 to 1.6 mg/mL. After that, the scavenging activity increased slowly with the increase of sample concentration. The IC_{50} value of PEPW80-1 was 2.0 mg/mL. The maximum value of PEPW80-1 reached 51.7% that of Vc.

3.4.3. *Ferric-reducing antioxidant power (FRAP)*

The antioxidant capacity of PEPW80-1 was shown in Figure 5c and compared with Vc. The reducing capacity ascended with increasing concentration from 0 to1.6 mg/mL. The maximum value of PEPW80-1 reached 57.8% that of Vc.

3.4.4. *In vitro immunomodulatory activity of PEPW80-1*

MTT assay was used to evaluate splenocyte proliferation

induced by Con A or PEPW80-1 *in vitro*. Spleen cells increased with the increase of sample concentration, and the polysaccharide significantly promoted the proliferation of splenocytes. At a lower concentration (25 μg/mL) PEPW80-1 could significantly enhance induced lymphocyte proliferation ($p < 0.01$) as compared with that of the normal control group (Figure 6).

4. Discussion

Polysaccarides, consisting 10 or/over 10 monosaccarides joined by glycosidic linkages, are considered as active compounds in plants and animals (*36-38*). Nowadays, many studies demonstrated that botanical polysaccharides had antioxidant activity (*2*) and immunomodulatory activity (*5*). As there was no study in polysaccharides of *P. emblica*, it attracted our interest to know if it had any polysaccharide with biological activities. PEPW80-1 was isolated and characterized from the pulp tissues of *P. emblica*, which contained rhamnose, arabinose and galactose (3.02:1.00:4.23).

Although antioxidant activity was usually associated with low molecular weight compounds such as phenols and carotenoids, increasing evidence indicated that polysaccharides from different sources might also possess strong antioxidant properties without concerns of cytotoxicity and could be developed as novel dietary antioxidants (*39*). Therefore, the DPPH radical, HOSC and FRAP assays were further evaluated for PEPW80-1.

It is well accepted that DPPH radical scavenging by antioxidants is due to their hydrogen-donating ability. The method of scavenging DPPH radical is based on the reduction of DPPH radical ethanol solution in the presence of a hydrogen-donating antioxidant, resulting in the formation of the non-radical form DPPH-H. DPPH radical is a stable radical and could accept an electron or hydrogen radical to become a stable diamagnetic molecule. Therefore, the antioxidant activity of a substance might be expressed as its ability in scavenging the DPPH radical. The results of DPPH radical scavenging test showed that the maximum value of PEPW80-1 reached 69.2% that of Vc and the IC_{50} values of PEPW80-1 was 0.5 mg/mL. PEPW80-1 had greater DPPH radical scavenging ability than polysaccharide from Panax japonicus (*40*) with a value IC_{50} of 3.0 mg/mL. These results indicated that the PEPW80-1 could act as an electron or hydrogen donator to scavenge DPPH radical.

Hydroxyl radical can easily cross cell membranes, readily react with most biomolecules including carbohydrates, proteins, lipids, and DNA in cells, and cause tissue damage or cell death (*41*). The IC_{50} value of PEPW80-1 was 2.0 mg/mL, which was less than the polysaccharide from *Panax japonicus* (5 mg/mL) (*40*). The maximum value of PEPW80-1 reached 51.7% that of Vc. The antioxidant mechanism might be due to the supply of hydrogen by the polysaccharide, which combines with radicals and forms a stable radical to terminate the radical chain reaction. The other possibility is that polysaccharide could combine with the radical ions which are necessary for radical chain reaction, and the reaction is terminated.

The FRAP assay treats the antioxidants contained in the samples as reductants in a redox linked colorimetric reaction, and the value reflects the reducing power of antioxidants. It has been reported that there was a direct correlation between antioxidant activity and reducing capacity (*42*). The antioxidant potential of PEPW80-1 was estimated by its ability to reduce the ferric complex to the ferrous complex. The maximum value of PEPW80-1 reached 57.8% that of Vc. It suggested that PEPW80-1 was electron donors and could react with free radicals to convert them into more stable products and terminate the radical chain reactions. Our experimental results demonstrated that there might be a direct correlation between antioxidant activity and reducing capacity.

Immunoregulation was one of the basic functions associated with polysaccharides (*43*). The spleen is the body's largest immune organ, capable of producing a large number of lymphocytes. Splenocyte proliferation is a crucial event in the activation cascade of both cellular and humoral immune responses. Immunomodulatory effect of the PEPW80-1 was investigated, and the results showed that PEPW80-1 could significantly induce lymphocyte proliferation at a low concentration (25 μg/mL), which is greater than the polysaccharides from the swollen culms of *Zizania latifolia* (200 and 400 μg/mL) (*44*).

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