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Polysaccharides from *Panax japonicus* C.A. Meyer and their antioxidant activities

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ABSTRACT

Polysaccharides named as PP1, PP2, PP3, PP4 and PP5 were extracted and isolated from the rhizomes of *Panax japonicus* C.A. Meyer, a well-known Chinese traditional medicine, by controlling the final concentration of solution to precipitate the polysaccharides. The molecular weight of polysaccharides was determined by HGPLC chromatography system. The monosaccharide composition was analyzed by Gas chromatography on an Agilent 6890A instrument using a DB-35MS column and flame-ionization detector. All of the polysaccharides were heteropolysaccharides and consisted of arabinose, glucose and galactose. The content of arabinose increased with the increasing of ethanol concentration and PP5 had the most arabinose content in these samples. IR spectra indicated obvious characteristic peaks of polysaccharide, the presence of uronic acids. Their antioxidant activities were evaluated by various established in vitro systems, including scavenging activity of superoxide anion, hydroxyl radical, ABTS and DPPH radicals. Both samples showed inhibitory effects on superoxide, hydroxyl, ABTS and DPPH radical. And PP5 shows more clearly and relatively stronger capacity than other polysaccharides on the protective effect of DNA damage.

1. Introduction

Panax japonicus C.A. Meyer, belongs to Araliaceae *Panax* family, has a bamboo-like long horizontally creeping rhizome and mainly grows in China, Japan and Korea (Morita et al., 1983). The rhizomes of *P. japonicus*, named king of herbs in traditional Tujia and Hmong medicine, have been used as a substitute for Ginseng roots in minority ethnic groups (He et al., 2012). It has been included in the Pharmacopeia of the People's Republic of China due to its various pharmacological activities, including anti-obesity, antiulcer, analgesic, expectorant, anti-inflammatory, antifatigue, antioxidant, anticancer and immunoregulation (Han, Zheng, Yoshikawa, Okuda, & Kimura, 2005; National Commission of Chinese Pharmacopoeia, 2010; Yamahara, Kubomura, Miki, & Fujimura, 1987). In recent years, saponins in this plant have been widely studied for their chemical properties and biological activities. However, a little attention was devoted to polysaccharides in *P. japonicas*.

Polysaccharides from natural sources are considered to be effective substances and possess marked immunological properties ranging from nonspecific stimulation of host immune system, resulting in anti-tumor and anti-viral effects, to antioxidant and anti-mutagenic activities (Kennedy, 1989; Sun, Liu, & Kennedy, 2010). Recently, polysaccharide from *P. japonius* has been reported to possess conspicuous effects on reticuloendothelial system activation (Ohtani, Hatono, Mizutain, Kasai, & Tanaka, 1989). Five polysaccharide samples were separated from *Rhizoma panacis japonici* by using different methods (Huang & Zhang, 2009). In our previous reports, two polysaccharides, consisted of glucose and galactose were isolated from *P. japonius* (Wang et al., 2012a).

As is known to all, uncontrolled production of oxygen-derived free radicals as mediators of inflammation and ischemia/reperfusion injury may damage numerous biological substances, including DNA, protein and lipid membranes in living cells (Cuzzocrea, Riley, Caputi, & Selvemini, 2001; Sahreen, Khan, & Khan, 2010), resulting in various diseases and disorders, such as cancer, cardiovascular diseases, rheumatoid arthritis, atherosclerosis and aging (Xu et al., 2009; Wu & Hansen, 2008). Antioxidants can provide protection cells against the damaging

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effects of reactive oxygen species (ROS) by scavenging free radicals (Zhang, Wang, Han, Zhao, & Yin, 2012). In recent years, there has been increasing interest in finding natural, effective and low cytotoxicity antioxidants because of the carcinogenicity of synthetic antioxidants (Guyton et al., 1991; Kimmel, Kimmel, & Frankos, 1986). It has been reported that polysaccharides isolated from plants have certain strong antioxidant abilities on free radicals and should be paid more attention to exploring them as novel potential antioxidants (Capek, Machová, & Turjan, 2009; Chen, Zhang, Jiang, Mu, & Miao, 2012; Qi et al., 2006). It is necessary to prepare different molecular weight polysaccharides have great influence on their biological activities (Zhang, Wang, Mo, & Qi, 2013).

The purpose of the present study was to isolate the different molecular weight polysaccharides from the rhizomes of *P. japonicus*, character their properties and investigate their antioxidant activities using various established in vitro systems.

2. Materials and methods

2.1. Materials

The *P. japonicus* were obtained from Enshi Tujia and Miao Autonomous Prefecture, China. m-Hydroxydiphenyl was from Acros Orgincs (Geel, Belgium). 2,2′-Azino-bis(3-ethylebenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,6-di-tert-butyl-4-methylphenol (BHT) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All of other reagents were of analytical grade.

2.2. Preparation of polysaccharides

The powdered material (250 g) was refluxed with 85% EtOH at 70 °C in a water bath for 3 h to remove substances which affected the color. After incubation, mixtures were centrifuged at 8000 r/min for 10 min. The insoluble residue was dried at 40 °C and extracted twice with distilled water (1:40, w/v) at 94 °C for 3.7 h (Wang, Chen, Jia, Tang, & Ma, 2012). The water extract was concentrated in a rotary evaporator and precipitated by anhydrous ethanol at 4 °C for 24 h. The precipitate was separated by centrifugation (10,000 rpm for 15 min) and air dried for 12 h. Protein was removed by Sevag method (Sevag, Lackman, & Smolens, 1938). The polysaccharide sample was dissolved in distilled water and ethanol was added to the solution at a final concentration of 40% to precipitate the polysaccharide (PP1) at 4 °C. Ethanol was added to the supernatant of 40% ethanol solution at a final concentration of 50% to precipitate the polysaccharide (PP2). Then, we obtained separately other polysaccharides (PP3, PP4 and PP5) at final ethanol concentrations of 60%, 75% and 90%.

2.3. High performance gel permeation chromatography analysis

The molecular weights of five kinds of polysaccharides were determined by high performance gel permeation chromatography (HPGPC) with a TSK-GEL G3000PWxl column (7.8 mm × 300 mm) and a refractive index detector (Shimadzu RID-10A). The eluent used was 0.2 M Na_2SO_4 in HPLC-grade water, with a flow rate of 0.5 mL/min. The column temperature was maintained at 40 °C. The molecular weight was estimated by reference to a calibration curve made from dextran standards. The data was recorded and processed by Shimadzu LC-Solution software.

2.4. Monosaccharide identification

Ten milligram polysaccharide was hydrolyzed in 5 mL of 2 M trifluoroacetic acid (TFA) at 100 °C for 6 h. After complete hydrolysis, the digested solution was evaporated under vacuum to dry. Then $NaBH_4$ (30 mg) and 2 mL distilled water were added to the hydrolyzed products, followed by acidification with acetic acid after incubation for 30 min. The solution was evaporated to dryness at 60 °C and 2 mL of 0.1% HCl–MeOH (v/v) was added, which was evaporated again to dryness. Then acetylation was carried out with 1:1 pyridine–acetic anhydride in water bath for 1 h at 90 °C. The monosaccharide composition was analyzed by Gas chromatography (GC) on an Agilent 6890A instrument using a DB-35MS column (30 m × 0.25 mm × 0.25 μm) and flame-ionization detector (FID). The operation was performed using the following conditions: column temperature was programmed from 130 °C (maintained for 1 min) to 170 °C at a rate of 6.5 °C/min, increased to 215 °C at a rate of 2.75 °C, and held for 7 min at 215 °C; the rate of N_2 carrier gas was 1.0 mL/min; injection temperature was 250 °C; detector temperature was 280 °C. Standards (D-glucose, D-xylose, D-galactose, L-rhamnose, D-mannose, and D-arabinose) with myo-inositol as the internal standard were prepared and subjected to GC analysis.

2.5. Chemical analysis of polysaccharides

Total sugar content of polysaccharide was measured by Phenol–Sulfuric acid methods (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was determined according to the method of Bradford (1976). The content of uronic acid of PJP was carried out by m-hydroxydiphenyl (Blunenkrantz & Asboe-Hansen, 1973).

2.6. IR spectroscopy

Polysaccharide was ground with spectroscopic grade KBr powder and then pressed into a 1 mm pellet for Fourier transform infrared (FT-IR) measurement by a Nicolet Nexus 5DXC FT-IR infrared spectrometer (Nicolet Co. Ltd., USA) in the frequency range 4000–500 cm^{-1} (Kumar, Joo, Choi, Koo, & Chang, 2004).

2.7. Antioxidant activity

2.7.1. Determination of scavenging activity on DPPH radical

The free-radical scavenging capacity of polysaccharides was analyzed by using DPPH test according to the method described by Li, Zhou, and Han (2006) with a minor modification. Reaction mixtures in a final volume of 4 mL contained 2 mL DPPH solution (0.2 mM in dehydrated alcohol) and various concentrations polysaccharides samples. Discolorations were measured at 517 nm after incubation for 30 min at 25 °C in the dark. BHTTHT was used as positive control. The inhibition percentage of DPPH radicals by the samples was calculated using the following equation: scavenging percentage activity on DPPH (%) = [1 − (Abs. of sample − Abs. of blank)/Abs. of control] × 100%.

2.7.2. Determination of scavenging activity on superoxide anion

The scavenging activities of related polysaccharides samples for superoxide anion were measured according to the method of Marklund and Marklund (1974) with some modifications. Briefly, 4.5 mL of 0.05 M Tris–HCl buffer (pH 8.2) was kept in water bath at 25 °C for 20 min. Then 1 mL of sample solution and 0.4 mL of 25 mM 1,2,3-phentriol were added to the reaction mixture and incubated at the same temperature for 5 min. Finally, the reaction system was quickly terminated by the addition of 1 mL HCl (8 mM). Ascorbic acid was used as positive control. The absorbance of resulting solution was measured spectrophotometriclly at 299 nm and the capability to scavenge superoxide anion radical was calculated according to the formula: scavenging effect (%) = [1 − (Abs. of sample − Abs. of blank)/Abs. of control] × 100%.

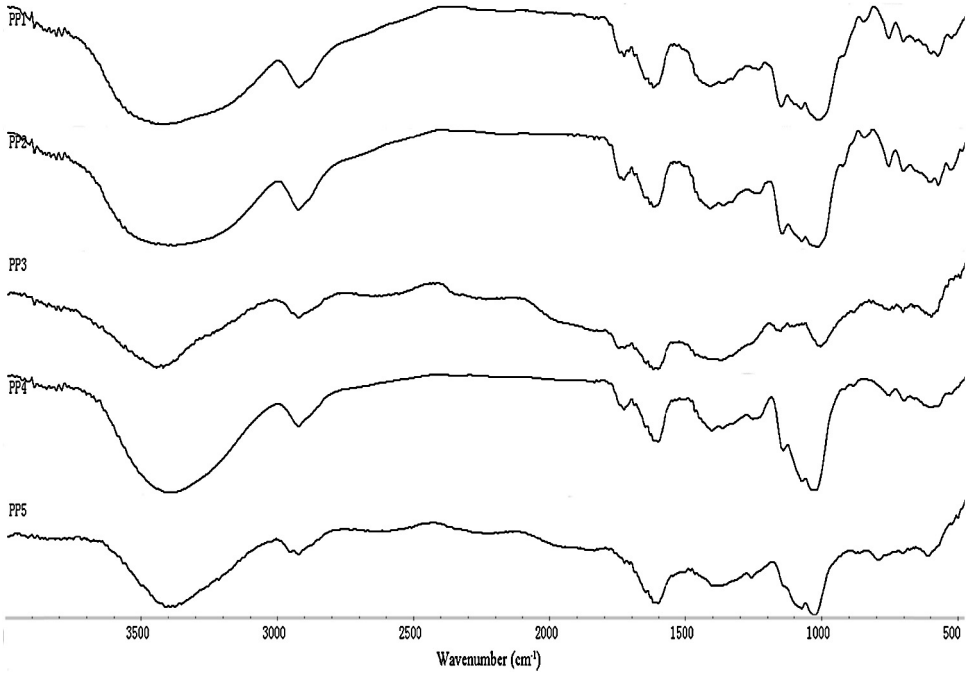

Fig. 1. FT-IR spectra of polysaccharides.

2.7.3. Determination of scavenging activity on hydroxyl radical

The capability to scavenge hydroxyl radical ($HO^{\bullet}$) was assessed according to the method reported by Sminoff and Cumbes (1989) with minor modifications. In the assay, 1.0 mL of 9 mM $FeSO_4$ and 1.0 mL of 9 mM salicylic acid (dissolved by alcohol) were added to 0.1 mL of sample solution at various concentrations. Afterwards, 1.0 mL of 8.8 mM H_2O_2 was added to reaction mixture and incubated for 30 min at 25 °C. Absorbance was then measured at 510 nm. Ascorbic acid was used for comparison. The capability of scavenging hydroxyl radical was calculated using the following equation: scavenging effect (%) = [1 − (Abs. of sample − Abs. of blank)/Abs. of control] × 100%.

2.7.4. Determination of scavenging activity on ABTS radical

This ABTS radical scavenging activity of the samples was assessed with a reported procedure (Wootton-Beard, Moran, & Ryan, 2011) with some modifications. ABTS radical cation ($ABTS^{\bullet+}$) was chemically produced by mixing 2.5 mL of ABTS solution (7 mmol/L) with 0.5 mL of potassium persulphate (15 mmol/L) and leaving the mixtures to incubate in the dark at room temperature for 12–16 h. After incubation, the $ABTS^{+}$ solution was diluted to an absorbance of 0.7 ± 0.01 at 734 nm. Then 0.2 mL of $ABTS^{+}$ solution was added to 0.05 mL of various concentrations of polysaccharide solution. After reacting for 6 min at 25 °C, the absorbance readings were taken immediately at 734 nm. BHT was included as a positive control. The scavenging activity on ABTS radical was calculated by the following equation: scavenging effect (%) = [1 − (Abs. of sample − Abs. of blank)/Abs. of control] × 100%.

2.8. Determination of DNA damage protective effect

DNA damage protection activities of polysaccharides were checked on pIC333 plasmid DNA, isolated from *Escherichia coli* DH5α by Fast plasmid mini kit. Plasmid DNA was damaged by H_2O_2 and UV treatment using the method of Russo et al. (2000) with some modifications. Rutin was included as a positive control. The electrophoresis was applied to separate different structural or conformational forms of plasmid DNA. Then, reaction mixture (10 μL) contained 3 μL of plasmid DNA, 5 μL of 5 mg/mL polysaccharide or 0.4 mg/mL Rutin, 1 μL of 10 mmol/mL H_2O_2 and 1 μL of tri-distilled water. The mixtures were located in super clean bench with ultraviolet lamp (20 W). The reactions were initiated by UV irradiation and last for 3 min at room temperature. After the irradiation, reaction samples along with gel loading dye (10×) were analyzed by electrophoresis on a 0.8% agarose horizontal slab gel in Tris–borate–EDTA buffer (45 mmol/L Tris–borate, 1 mmol/L EDTA) at pH 8 for 35 min (100 V) (Biso et al., 2010).

Table 1
The chemical composition and characterizations of all the samples.

Samples	Total sugar (%)	Uronic acids (%)	Neutral sugar (mole ratio)[a]		
			Ara	Gal	Glc
PP1	98.01	27.03	0.51	1.00	5.80
PP2	95.95	27.71	0.34	1.00	6.20
PP3	97.01	19.33	0.32	1.00	1.31
PP4	93.88	25.62	0.44	1.00	1.01
PP5	97.56	23.69	2.25	1.00	2.82

[a] Gal: galactose; Glc: glucose; Ara: arabinose.

3. Results and discussion

3.1. Polysaccharide composition and molecular weight

Polysaccharides were graded by ethanol and obtained 40%, 50%, 60%, 75% and 90% of five kinds of polysaccharides which respectively named PP1, PP2, PP3, PP4 and PP5. The yields of these five samples were 42.2%, 21.6%, 8.7%, 12.3% and 4.9% in removed protein polysaccharide. No proteins existed in the five polysaccharides. The chemical composition and characterizations of all the samples were summarized in Table 1. The table shows that PP1 had the most total sugar content and PP4 had the least in these five polysaccharides. The composition of uronic acids in PP2 was the most in all samples, and that in PP3 was the least. According to the analysis of monosaccharide using GC, all of the polysaccharides were heteropolysaccharides and consisted of arabinose, glucose

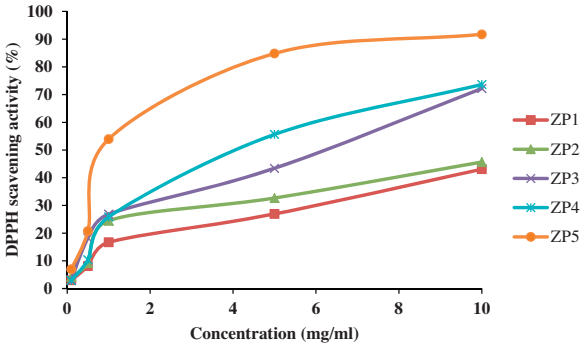

Fig. 2. Scavenging effects of polysaccharides on DPPH.

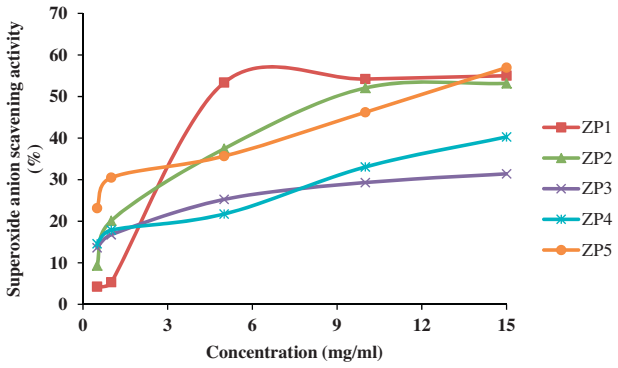

Fig. 3. Scavenging effects of polysaccharides on superoxide anion.

and galactose. Glucose was the main sugar unit among these five samples. The content of arabinose increased with the increasing of ethanol concentration and PP5 had the most arabinose content in these samples. The HPGPC system was used to determine molecular weight of polysaccharide. The equation of the standard curve was: Log Mw = 0.3362*t* + 9.1663 (where Mw represents the molecular weight, while *t* represents retention time) with a correlation coefficient of 0.9999. Results showed that the five polysaccharides were not homogenous compounds and contained some polysaccharides with different molecular weights. The Weight Average Molecular Weight (Mw) of PP1, PP2, PP3, PP4 and PP5 were 1.09×10^5, 5.27×10^4, 3.75×10^4, 2.77×10^4 and 4.81×10^3.

3.2. IR spectra analysis of polysaccharides

As shown in Fig. 1, FT-IR spectrum indicated obvious characteristic peaks of polysaccharide, the presence of uronic acids. The infrared spectra of polysaccharides all displayed a broad stretching intense characteristic peak at around 3600–3200 cm^{-1}, which was attributed to the hydroxyl groups stretching vibration. The weak peak toward 3000–2800 cm^{-1} was due to the C—H stretching vibration. In addition, two stretching peaks at 1735 cm^{-1} and 1625 cm^{-1} suggested the presence of C=O and COO^- (Gnanasambandam & Proctor, 2000). And the absorptions around 1735, 1625 and 1410 cm^{-1} in the IR spectra indicated the presence of uronic acids. Two stretching peaks toward 1080 and 1155 cm^{-1} suggested the presence of C—O bonds.

3.3. Antioxidant activity

3.3.1. Scavenging effect on DPPH radical

The DPPH free radical, a stable radical with purple color and a maximum absorption at 517 nm, has been widely used as a tool to evaluate the free radical scavenging activities of antioxidants (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004). When DPPH encounters antioxidant scavengers, its purple color would fade rapidly because it was changed to the non-radical form DPPH-H (Yamaguchi, Takamura, Matoba, & Terao, 1998). Fig. 2 shows the DPPH radical scavenging activity of the five samples. The results indicated that the five samples exhibited very low radical scavenging activity at the concentration of 0.1 mg/mL. Compared to these samples, ZP5 has the strongest scavenging activity on DPPH at every concentration point. The scavenging activities of ZP5 increased significantly with the increase of sample concentration ranging from 0.5 to 1 mg/mL. In addition, the ZP3 and ZP4 had almost the same activity at 10 mg/mL. At the concentration of 10 mg/mL, the scavenging activities were 43.07%, 45.66%, 72.18%, 73.64% and 91.73% for ZP1, ZP2, ZP3, ZP4 and ZP5, respectively. The scavenging activity of positive control was 56.29% and 80.71% at the concentration of 0.2 mg/mL and 0.8 mg/mL, respectively. Compared with these polysaccharides, BHT was more effective for scavenging DPPH. The results suggest that all the polysaccharides possessed scavenging activities on DPPH in a concentration-dependent manner, and especially ZP5 had much stronger antioxidant activity. With the decrease on molecular weights of polysaccharides, the scavenging activities on DPPH gradually increased.

3.3.2. Superoxide radical scavenging activity assay

As a highly toxic species, superoxide anion radical is generated first of all the reactive oxygen species by numerous photochemical and biological reactions. Although it is a relatively weak oxidant, it can form stronger reactive oxidative species which may induce oxidative damage in lipids, proteins and DNA such as lipid peroxidation and cellular damage (Fan, Li, Deng, & Ai, 2012; Korycka-Dahl & Richardson, 1978; Wickens, 2001). Therefore, the scavenging activity of superoxide anion radicals is very important to antioxidant work.

The effects of superoxide radical scavenging activities of five samples were given in Fig. 3. Notably, ZP1 presented higher scavenging activity than other samples at the concentration ranging from 3 to 14 mg/mL. Interestingly, ZP5 exhibited relatively stronger superoxide radical scavenging activity than other samples at the lower doses (0.5–3.0 mg/mL), whereas the radical scavenging activity of ZP5 was weaker than that of ZP1 and ZP2 at the concentration ranging from 4 to 13 mg/mL. The scavenging activities of ZP3 and ZP4 increased slowly with the increase of sample concentration. The scavenging effect of five samples on superoxide anion followed the order: ZP5, ZP1, ZP2, ZSP4, ZP3 and were 56.96%, 55.01%, 53.18%, 40.28% and 31.41% at the concentration of 15 mg/mL, respectively. When the concentration of ascorbic acid was at 0.8 mg/mL, its scavenging activity has already reached 63.36%. Polysaccharides with special conformations, hydrogen in O—H bonds could be easily liberated and thus could stabilize superoxide anion. The mechanism of polysaccharide on scavenging superoxide anion may be related to the dissociation energy of O—H bond (Jin et al., 2012).

3.3.3. Scavenging activity of hydroxyl radical

Among the reactive oxygen species, hydroxyl radical is the most harmful free radical, generally accepted as a highly potent oxidant, and could induce severe damage to adjacent biomolecules (Li, Liu, Fan, Ai, & Shan, 2011). Hydroxyl radical was generated by the reaction of Fe(II) complex with hydrogen peroxide in the presence of salicylic acid which has the ability to absorb hydroxyl radical to bring coloring material. Hydroxyl radical scavenger competed hydroxyl radical with salicylic acid, which pared coloring material down. Fig. 4 depicts the scavenging activity of hydroxyl radical of five kinds of polysaccharides from *P. japoniuas*. The scavenging effect of polysaccharides increased with increasing sample concentration. The scavenging activities on hydroxyl radical of samples (ZP1, ZP5) were relatively higher than that of others (ZP2,

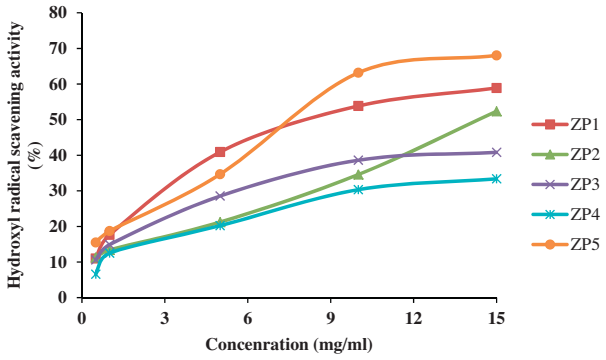

Fig. 4. Scavenging effects of polysaccharides on hydroxyl radical.

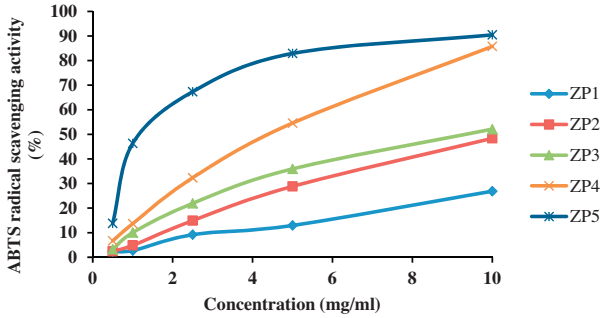

Fig. 5. Scavenging effects of polysaccharides on ABTS radical.

ZP3, ZP4) at the same dose. The scavenging activity of ZP4 was weaker than that of other polysaccharides and increased slowly with the increase of sample concentration. Compared ZP1 and ZP5, ZP5 had a higher scavenging ability than ZP1 at the concentration above 7.5 mg/mL. And the percentage scavenging of ascorbic acid ranged from 13.95% to 99.28% at the concentration from 0.1 to l mg/mL. There are two types of antioxidant mechanisms: suppression against hydroxyl radical generation and cleaning the hydroxyl radical generated. Researchers have suggested that both the mechanisms might be associated with the hydroxyl radical scavenging activity of polysaccharide (Wang, Zhang, Zhang, & Li, 2008).

3.3.4. *ABTS radical scavenging activity*

$ABTS^+$ assay is often used in measuring the total antioxidant power of a potential antioxidant and can also be used as an index to reflect the antioxidant activity of the test samples (Luo et al., 2010). The scavenging activities of samples on ABTS free radicals are shown in Fig. 5. The ABTS radical scavenging abilities of polysaccharide samples increased gradually as time extends. Moreover, ZP5 exhibited the highest scavenging effect on ABTS radicals among all samples, followed by ZP4, ZP3, ZP2 and ZP1. The ABTS radicals scavenging increased from 54.59% to 85.77%, when the concentration of ZP4 increased from 5 to 10 mg/mL. However, BHT could Exhibit 79 .22% ABTS scavenging activity at a low concentration of 0.02 mg/mL. The results indicated that ZP4 and ZP5 had strong scavenging activities on ABTS radicals and could be explored as potential antioxidants.

3.3.5. *Protective effect of polysaccharides on DNA damage*

The protective effect of *P. japonicus* polysaccharides on H_2O_2 + UV-induced damage was studied on pIC333 plasmid DNA. DNA migration assay is a sensitive biomarker of the DNA damage (Tepe, Degerli, Arslan, Malatyali, & Sarikurkcu, 2011). As can be seen from the Fig. 6, DNA derived from pIC333 plasmid showed bands on agarose gel electrophoresis (lane 8). The UV irradiation of DNA with

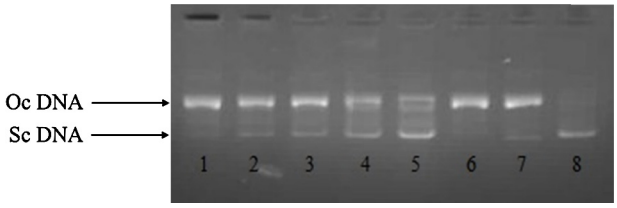

Fig. 6. Agarose gel electrophoretic pattern showing forms of plasmid pIC333. Lanes 1–5, H_2O_2 + UV treated with PP1, PP2, PP3, PP4 and PP5, respectively; lane 6, H_2O_2 + UV treated without polysaccharide; lane 7, H_2O_2 + UV treated with Rutin; lane 8, untreated DNA (control).

H_2O_2 (lane 6) resulted in the cleavage of supercoiled circular DNA (Sc DNA) to open circular form (Oc DNA) on the agarose gel (Kumar & Chattopadhyay, 2007). Lanes 1–5 revealed that the addition of polysaccharides to the reaction mixture conferred the protection to the damage of native Sc DNA. When the polysaccharide of *P. japonicus* was added to the reaction mixture, smear Sc DNA could be observed on the agarose gel. Lane 5 shows more clearly Sc DNA band than lanes 1–4, which suggested ZP5 had a relatively stronger capacity than other polysaccharides. The positive control (lane 7), ZP2 and ZP3 almost had the same protective effect. These results obtained from the protective effect on DNA damage indicated a correlation with the antioxidant potential of the polysaccharides.

4. Conclusions

The researches of natural polysaccharides have attracted a lot of attention because of their abundance in resources and non-toxicity. This article mainly related to the isolation of the antioxidant activities polysaccharides from *P. japonicus*. According to the results stated above, it could be concluded that we obtained five different Mws polysaccharides. Glucose was the main sugar unit among these five samples. The content of arabinose increased with the increasing of ethanol concentration and ZP5 had the most arabinose content in these samples. In vitro antioxidant activities studies indicated that the polysaccharides all exhibit activity in scavenging free radicals and protective effect on DNA damage. Compared to the positive, all the tested samples showed lower inhibitory effects on antioxidant activities. From the viewpoint of molecular weight, the lower Mws polysaccharides possessed stronger scavenging of polysaccharides on DPPH and ABTS radical. The lowest Mws polysaccharide ZP5 had the strongest inhibitory effects almost at every determined concentrations on DPPH and ABTS radical. Different Mws had different scavenging effects on hydroxyl radical and superoxide radical. This study indicated that the molecular weight might be a significant factor on antioxidant capacity. However, to know more about the polysaccharides of *P. japonicas*, the relationship of structures and various activities will be planned for further investigation. Meanwhile, studies of molecular modification of *P. japonicas* polysaccharides are underway to improve and amplify the biological activities.

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