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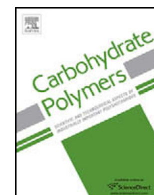

Antioxidant activity and characterization of antioxidant polysaccharides from pine needle (*Cedrus deodara*)

Wei-Cai Zeng[a], Zeng Zhang[b], Li-Rong Jia[a,*]

[a] College of Light Industry, Textile and Food Engineering, Sichuan University, Chengdu 610065, PR China
[b] College of Life Sciences, Sichuan University, Chengdu 610065, PR China

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A B S T R A C T

A novel antioxidant polysaccharide (APC) was isolated and characterized from pine needles of *Cedrus deodara* with the evaluation of its *in vitro* antioxidant activity. According to gel filtration chromatography, high performance size exclusion chromatography, gas chromatography–mass spectrometry, partial acid hydrolysis, periodic acid oxidation, Smith degradation and methylation analysis, APC was observed to be an acidic heteropolysaccharide (composed of glucose, arabinose, mannose and xylose in a molar ratio of 45.84:1:2.35:1.73) with the molecular weight of 1.53×10^4 Da, and the backbone was mainly composed by glucose, mannose and xylose in the form of $(1 \rightarrow 4)$ linked. Meanwhile, APC exhibited the remarkable antioxidant activity to scavenge free radicals and inhibit the oxidative injury of DNA and cells. The present results suggested that APC could be a potential antioxidant agent for preparing functional foods and nutraceuticals applied in food and pharmaceutical industries.

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1. Introduction

Reactive oxygen species (ROS) generated in aerobic organisms during respiration process, such as hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), may be necessary for normal function of cells at physiological levels (Page, Moser, Chen, & Dutton, 1999). But, the excessive ROS are harmful to cells, due to their strong attacking capacity to lipids, proteins and deoxyribonucleic acid (DNA), which can cause various cellular damages and diseases, such as oxidative damage of DNA, cardiovascular disease and neurological disease (Martínez-Cayuela, 1995; Fraser, 2011; Gil del valle, 2011). In the last decade, huge interests had received the great attention and had been studied extensively to identify free radical scavengers or antioxidants, which could reduce risks of oxidative damage and several cardiovascular diseases. Although the synthetic antioxidants, such as propyl gallate (PG) and butylated hydroxytoluene (BHT), could inhibit the oxidative damage effectively, their potential toxic in human body was being considered and worried (Carocho, & Ferreira, 2013). Thus, many efforts had been made to find the novel and safe antioxidants from natural source (Ng, Liu, & Wang, 2000; Podsędek, 2007; Karre, Lopez, & Getty, 2013).

Polysaccharides, one essential biomacromoleules of life activities, are usually composed of various monosaccharides linked with different glucosidic bonds, which widely exist in plants, animals and microorganism. Being associated with proteins and polynucleotides, polysaccharides participate in cell adhesion, signal recognition, molecular recognition and cell–cell communication, and also play important roles in the immunization, reproduction and blood systems (Dwek, 1996; Gilbert, Knox, & Boraston, 2013). Recently, many bioactive polysaccharides obtained from different natural source were observed to exhibit various biological activities, such as immunopotentiation, reducing the blood glucose and blood triglycerides, antitumor, anticoagulant, antiirradiation and antioxidant activities, which have attracted much attention in the field of biochemistry and pharmacology (Jin, Zhao, Huang, Xu, & Shang, 2012; Cao, 2013). Furthermore, a lot of studies had been made to elucidate the relationship between structures and biological effects of polysaccharides, and the accumulated evidence demonstrated that structure of polysaccharides had the significant influence to their biological activities (Yang & Zhang, 2009; Sun, 2011).

Cedrus deodara, commonly named Himalayan cedar, belongs to *Pinaceae* and widely grows in Asia, such as China, India, Japan and Korea. With the significant nutritional and pharmaceutical effects, pine needles of *C. deodar* have been widely used in food industry to

* Corresponding author at: College of Light Industry, Textile and Food Engineering, Sichuan University, Chengdu, Sichuan 610065, PR China. Tel.: +86 28 85405236; fax: +86 28 85405137.
E-mail address: lrjia@sina.com (L.-R. Jia).

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produce beverage, and in herbal medicine to treat tic, fever, cough, bronchitis and tuberculosis (Fang, Quan, & Cai, 2010; Chaudhary, Ahmad, & Mazumder, 2011; Bai, Shi, Liu, & Li, 2012). Recently, it has been reported that water-extract of *C. deodara* pine needles exhibited the remarkable antibrowning, antioxidant and antibacterial activities, which was due to its high content of phenolic compounds (Zeng et al., 2011; Zeng, He, Sun, Zhong, & Gao, 2012). Furthermore, its essential oil was observed to possess the excellent antioxidant and antimicrobial capacity (Zeng, Zhang, Gao, Jia, & He, 2012). Therefore, pine needles of *C. deodar* might have the potential value to be utilized as a novel nutraceuticals to protect human health.

To the best of our knowledge, the investigation about the characterization and corresponding antioxidant activity of polysaccharides from *C. deodara* pine needles is rather limited. Therefore, efforts have been paid to characterize a novel antioxidant polysaccharide from *C. deodara* pine needles, and its antioxidant activities were also determined detailedly in present study. Furthermore, we attempted to observe its protective effects on the oxidative injury of DNA and cells induced by hydrogen peroxide.

2. Materials and methods

2.1. Materials and reagents

Pine needles of *C. deodara* were collected from Chengdu of China in July, 2012. The plant was initially identified by the morphological features, and morphological data were kept in the Department of Biology, Sichuan University. A voucher specimen was dried and preserved at the Department of Food Engineering, Sichuan University. *C. deodara* pine needles were washed and dried at 45 °C for 12 h, and then were crushed into powders (about 60 granularities) with a mixer (JYL-350, Jiuyang Co., Ltd., China), and finally stored under vacuum.

2,2′-Azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS), pyridine, inositol, acetic anhydride, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butyl hydroxy anisd (BHA), nitro blue tetrazolium (NBT), dihydronicotineamidadenine dinucleotide (NADH), phenazine methosulfate (PMS), ethylene diamine tetraacetic acid (EDTA), trifluoroacetic acid (TFA) and thiobarbituric acid (TBA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Plasmid pBR322 DNA was purchased from Takara Bio-medicals (Tokyo, Japan). A Chinese hamster lung fibroblast cell line (V79 cells) was purchased from Chengdu Biological Institute (Chengdu, China). The solvents for high performance size exclusion chromatography (HPSEC) and gas chromatography–mass spectrometry (GC–MS) were of chromatographic purity. All other reagents were of analytical grade. The water used in all test was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Extraction of crude polysaccharides

The pine needle powders (2000 g) were added to 5000 mL of distilled water with continuously stirring at 85 °C for 45 min. Then, the mixture was filtered and the extraction procedure repeated twice. Thereafter, all supernatants were condensed using a rotary evaporator (R-II, Kejinyu, Co. Ltd., Zhengzhou, China) at 45 °C under vacuum. Bing treated with Sevage reagent and dialyzed (MWCO 5000, Sigma–Aldrich, USA), crude polysaccharides were precipitated with 5-fold volumes of absolute alcohol, and then were prepared by centrifugation and freeze-drying. The yield of crude polysaccharides was 3.58% (3.58 g crude polysaccharides/100 g dried pine needle powders).

2.3. Purification and characterization of antioxidant polysaccharides

2.3.1. Isolation and purification

Crude polysaccharides (10 g) were separated on the DEAE-cellulose 52 column (3 cm × 50 cm), Sephadex G-50 gel filtration column (3 cm × 75 cm) and Toyopearl HW-65F column (3 cm × 70 cm), and eluted using a linear gradient of 0–1 M NaCl solution at a flow rate of 0.2 mL/min to arrive at homogenous preparation. The polysaccharides obtained at this stage were designated as "APC". The total saccharide content and quantification of uronic acid of APC were determined according to the phenol-sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and the vitriol-carbazole method (Yang et al., 2008), respectively.

2.3.2. Determination of homogeneity, molecular weight and monosaccharide composition

According to the analytical methods in our previous study (Zeng, Zhang, Gao, Jia, & Chen, 2012), the homogeneity and molecular weight of APC were determined by high performance size exclusion chromatography (HPSEC) using an Agilent 1100 HPLC system (Agilent Technologies, Ltd., CA, USA) and a size exclusion chromatography (SEC) column (TSK gel Super SW 2000, 4.6 mm × 300 mm i.d. with a particle size of 4 μm, Tosoh, Tokyo, Japan), and the monosaccharide composition of APC was analyzed by gas chromatography–mass spectrometry (GC–MS).

2.3.3. Partial acid hydrolysis, periodic acid oxidation, Smith degradation and methylation analysis

The structure properties of APC, such as the link model and sequence of monosaccharide, was characterized by using the analytical tests of partial acid hydrolysis, periodic acid oxidation, Smith degradation and methylation analysis according to the methods in our previous study (Zeng, Zhang, Gao, Jia, et al., 2012).

2.4. Evaluation of the free radical scavenging activity

The free radical (ABTS, DPPH, superoxide and hydroxyl radicals) scavenging activity of APC was evaluated according to the detailed methods in our previous study (Zeng, Zhang, Gao, Jia, et al., 2012). APC was dissolved in distilled water at different concentrations (0.25–4 mg/mL) for the measurements.

2.5. Protection on the oxidative damage of DNA induced by hydrogen peroxide

The protective capacity of APC on the oxidative damage of DNA induced by hydrogen peroxide was determined as the procedure described by Yeung et al. (2002) with some modifications. Briefly, 3 μL of phosphate buffer (50 mM, pH 7.4) containing 0.5 μg of pBR322 DNA, 3 μL of EDTA–$FeSO_4$ solution (2 mM) and 2 μL of APC solution at various concentrations (20–320 μg/mL) were mixed. Then, 4 μL of 30% H_2O_2 were added and the mixture was incubated in water bath at 37 °C for 1 h, which was conducted in an Eppendorf tube. When the reaction was completed, the mixture was subjected to 1% agarose gel electrophoresis. DNA bands (supercoiled, linear, and open circular) were stained with ethidium bromide and quantified by scanning the intensity of bands with quantity one program (version 4.2.3, BioRad Co.). Protective effect on DNA was based on the increase or loss percentage of supercoiled monomer, comparing with control. To avoid the effects of photoexcitation of samples, all assays were done in the dark.

2.6. Protection on the oxidative injury of cells induced by hydrogen peroxide

2.6.1. Cell culture

V79 cells were maintained in RPMI 1640 supplemented with 5% fetal bovine serum, 10% horse serum, 100 U/mL penicillin G, 80 U/mL streptomycin and 10 mM Hepes. They were grown at 37 °C in a humidified atmosphere of 5% CO_2. The culture medium was changed every 2 days and the cells were tripsinized when confluence reached approximately 80%. For further assay, the cells were seeded in 96 microplates at a density of 1×10^5 cells/mL (100 μL for each well), and the culture media were changed every 2 days.

2.6.2. Cell viability assay

The protection of APC on the oxidative injury of V79 cells induced by H_2O_2 was evaluated by measuring the cell viability using MTT test.

When V79 cells were plated in collagen-coated 96-well microplates at a density of 1×10^5 cells/mL, 20 μL of culture medium containing various concentrations of APC (20–320 μg/mL) was used in cell incubation for 2 h. Afterwards, medium was removed and washed three times with phosphate buffered saline (PBS, pH = 7.4). Then, fresh medium containing H_2O_2 (0.15 mM) was added and incubated for 2 h. Subsequently, the medium was removed and fresh medium containing MTT (0.5 mg/mL) was added and incubated for 4 h. After removing of culture medium, cells were lysed by using DMSO. The absorbance of mixture was measured at 570 nm. Cells cultured without APC and H_2O_2 were used as control. All incubations were carried out at 37 °C, 5% CO_2 and 95% relative humidity in the cell incubator. The protective effect of APC was described using the cell viability which was calculated as $(A_{\text{sample } 570}/A_{\text{control } 570}) \times 100\%$. Blank wells contained only culture medium were used for background correction.

2.7. Statistical analysis

Data were subjected to statistical analysis using SPSS (version 15.0 for Windows, SPSS Inc., CO, USA). The data were reported as mean ± standard deviation (SD). Differences were considered significant at $P < 0.05$.

3. Results

3.1. Isolation and purification of APC

The ion exchange chromatography (IEC) and gel filtration chromatography (GFC) was employed for the isolation and purification of antioxidant polysaccharides from *C. deodara* pine needles. As shown in Fig. 1A, crude polysaccharides were subjected to a DEAE-cellulose 52 column to yield three distinct peaks (fraction-1, fraction-2 and fraction-3). With the strong biological activity (58.79% and 43.62% for ABTS and DPPH radicals scavenging activity with the testing concentration of 5 mg/mL), the second peak (fraction-2) was further separated on a Sephadex G-50 column (Fig. 1B) to obtain two peaks (Fraction-a and Fraction-b). The second peak (fraction-b) showed prominent biological activity (74.56% and 46.82% for ABTS and DPPH radicals scavenging activity with the testing concentration of 2 mg/mL) and was further purified on a Toyopearl HW-65F column (Fig. 1C) to obtain APC with the color of white. The ultraviolet spectra of APC revealed that

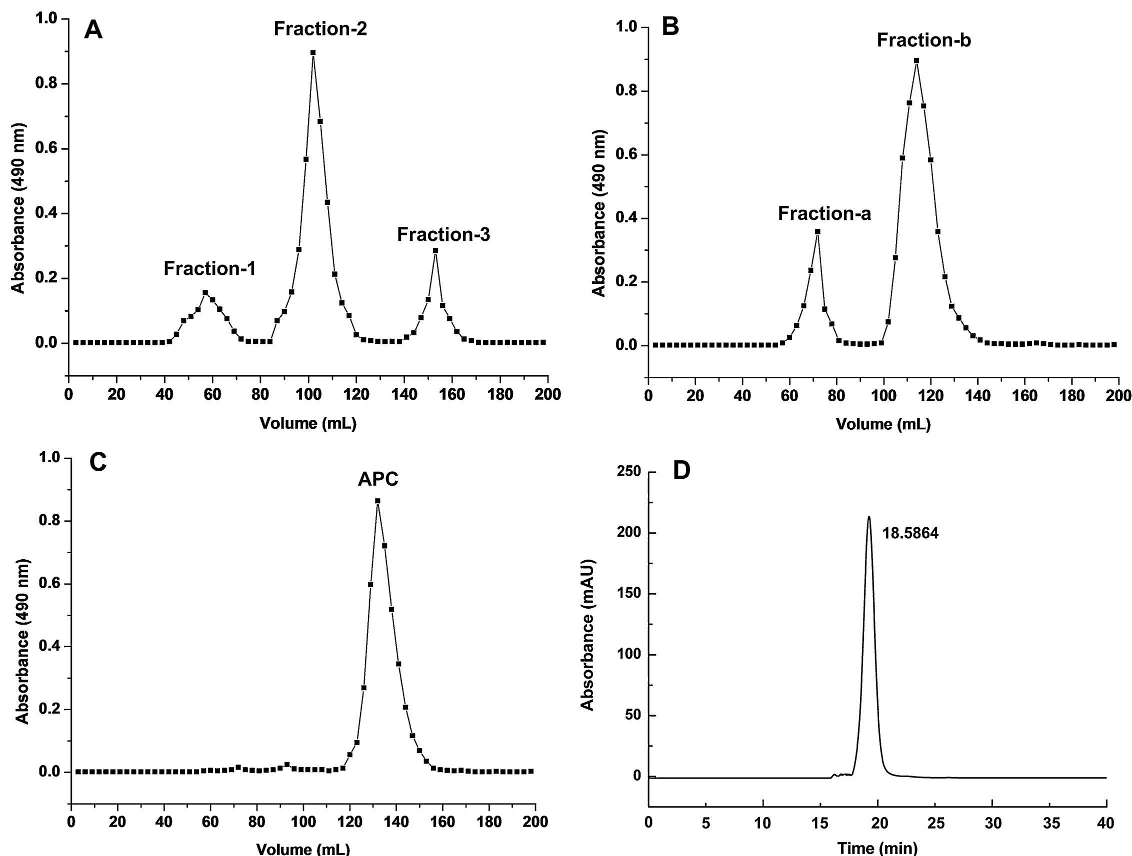

Fig. 1. Isolation and purification of APC. (A) Chromatogram of crude polysaccharides by DEAE-cellulose 52 chromatography; (B) chromatogram of fraction-2 by Sephadex G-50 chromatography; (C) chromatogram of fraction-b by Toyopearl HW-65F chromatography; and (D) HPSEC chromatogram of APC.

Table 1
Analysis result of partial acid hydrolysis.

Fractions	Molar ratios			
	Glucose	Arabinose	Mannose	Xylose
APC-a[a]	6.37	1	0.54	0.23
APC-b[b]	35.78	1	1.58	1.35
APC-c[c]	33.56	1	5.71	4.53
APC-d[d]	31.69	1	2.16	1.43

[a] Precipitation after hydrolysis.
[b] Precipitation in the sack.
[c] Supernatant in the sack.
[d] Fraction out of sack.

there was no absorption at 280 or 260 nm, which indicated that APC contained no protein or nucleic acid. The total saccharide and uronic acid contents of APC were 97.85% and 16.32%, respectively.

3.2. Characterization and structural elucidation of APC

3.2.1. Homogeneity, molecular weight and monosaccharide composition determination

High performance size exclusion chromatography (HPSEC) analysis (Fig. 1D) indicated that APC was a homogeneous polysaccharide with a high purity, and its equivalent dextran molecular weight was estimated to 1.53×10^4 Da based on the equation of the standard curve made with a group of dextran standards. Furthermore, according to the analysis of monosaccharide using GC–MS, APC was a heteropolysaccharide and consisted of glucose, arabinose, mannose and xylose, with a molar ratio of 45.84:1:2.35:1.73.

3.2.2. Partial acid hydrolysis

Four fractions (APC-a, APC-b, APC-c and APC-d) were obtained through partial acid hydrolysis. Each fraction was subjected to GC–MS analysis and the results were shown in Table 1. Results indicated that glucose, mannose and xylose might be the backbone structure of APC, and glucose and arabinose might be the branched structure of APC. Glucose might be the terminal residues of backbone and branched.

3.2.3. Periodate oxidation and Smith degradation

Results of periodate oxidation showed that 1 mol of sugar residue consumed 0.68 mol of periodate and produced 0.13 mol of formic acid, indicating the presence of a few of monosaccharide that were (1→) linked or (1 → 6) linked. The fact that the amount of periodate consumption was more than the amount of formic acid (0.13 mol × 2) demonstrated that there were other linkage types that could not produce formic acid, such as (1 → 3) or (1 → 3,6).

The periodate-oxidized products were fully hydrolyzed and analyzed by GC–MS after periodate oxidation (Table 2). The predominant presence of glycerol demonstrated that the linkages of backbone were (1→) linked, (1 → 6) linked, (1 → 2) linked, (1 → 2,6) linked that could be oxidized to produce glycerol. The presence of erythritol indicated that the (1 → 4) linked and (1 → 4,6) linked were present in the backbone of APC. All the analytical results of partial acid hydrolysis, periodate oxidation and Smith degradation indicated that glucose, mannose and xylose were (1→) linked, (1 → 4) linked, and (1 → 6) linked in the backbone of APC, and glucose and arabinose distributed in branches were (1 → 3) linked, (1 → 4) linked, and (1 → 2) linked.

3.2.4. Methylation analysis

The fully methylated APC was hydrolyzed with acid and analyzed by GC–MS. As shown in Table 3, four methylation product, such as 2,3,4,6-Me_4-Glu, 2,4,6-Me_3-Glu, 2,3,6-Me_3-Glu and 2,3-Me_2-Glu, were determined with a molar ration of 1:5.85:23.56:3.23, which suggested that APC possessed four kinds of linkages, such as (1→) linked, (1 → 3) linked, (1 → 4) linked and (1 → 4,6) linked, and (1 → 4) linked was the main linkages.

According to the analytical results of partial acid hydrolysis, periodate oxidation, Smith degradation and methylation analysis, the structural properties of APC might be concluded. APC was an acidic heteropolysaccharide; the backbone of APC was mainly composed by glucose, mannose and xylose in the form of (1 → 4) linked; a few of branched structure was present in APC; a few of (1→) linked, (1 → 3) linked and (1 → 4,6) linked were also present in APC; glucose was the major components of APC; part of arabinose was distributed in branches.

3.3. Free radical scavenging activity of APC

As shown in Fig. 2, APC exhibited the remarkable antioxidant activity to scavenge ABTS, DPPH, superoxide and hydroxyl radicals, which were concentration-dependent. The ABTS scavenging ratio was in the range of 37.58–86.78% and the half-effective concentration (EC_{50}) was estimated at 0.64 ± 0.15 mg/mL (Fig. 2A), while the EC_{50} of BHA was 5.31 ± 0.42 mg/mL. Meanwhile, APC scavenged 13.56–64.78% of DPPH radicals (Fig. 2B) and its EC_{50} was 2.89 ± 0.38 mg/mL, which was lower than that (4.86 ± 0.49 mg/mL) of BHA. In addition, APC was also observed to possess the strong ability to scavenge superoxide radical (Fig. 2C). The scavenging ratio was 33.79% at 0.25 mg/mL of APC, and reached 76.12% when the concentration increased to 4 mg/mL, with the EC_{50} of 0.66 ± 0.11 mg/mL (EC_{50} of BHA was 5.86 ± 0.79 mg/mL). Furthermore, as present in Fig. 2D, APC showed the remarkable ability to scavenge hydroxyl radical with the scavenging range of

Table 2
Analysis result of Smith degradation.

Fractions	Molar ratios					
	Glucose	Arabinose	Mannose	Xylose	Glycerol	Erythritol
Full acid hydrolysis	45.84	1	2.35	1.73	–	–
Out of sack	6.75	2.31	2.01	1.35	18.76	5.36
Supernatant in sack	2.36	1.21	–	–	+	1.13
Precipitation in sack	1.57	1.12	–	–	1.25	+

Table 3
Results of methylation analysis.

Methylation product	Molar ratio	Mass fragments (*m*/*z*)	Type of linkage
2,3,4,6-Me_4-Glu	1	43, 71, 101, 117, 129, 145, 161, 205	1→
2,4,6-Me_3-Glu	5.85	43, 71, 87, 101, 129, 161, 233	1→3
2,3,6-Me_3-Glu	23.56	45, 86, 101, 113, 129, 147, 174, 235, 245	1→4
2,3-Me_2-Glu	3.23	43, 55, 87, 99, 129, 142, 159, 201, 263	1→4,6

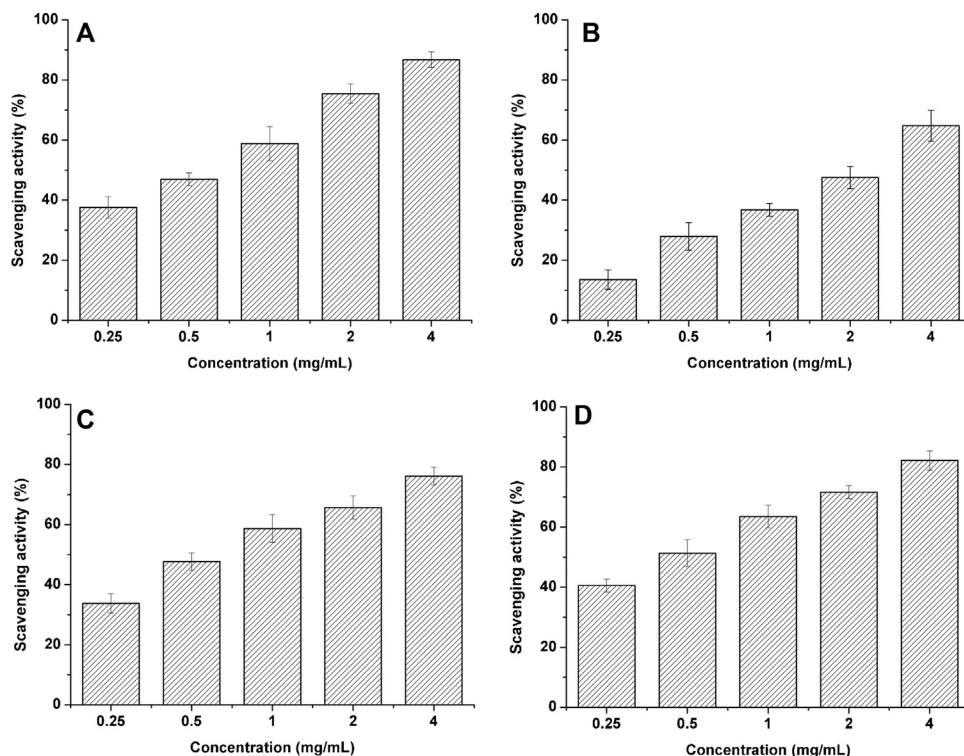

Fig. 2. Free radical scavenging activity of APC. (A) ABTS radical; (B) DPPH radical; (C) superoxide radical; and (D) hydroxyl radical.

40.58–82.13% and its EC_{50} was 0.49 ± 0.05 mg/mL (EC_{50} of BHA was 3.23 ± 0.28 mg/mL).

3.4. Protective effect of APC on the oxidative injury of DNA and cells

A free radical-induced plasmid pBR322 DNA breaks *in vitro* system was used to observe the protective effect of APC on DNA damage. With the attack of hydroxyl radical from H_2O_2 and Fe^{2+}, supercoiled plasmid DNA was broken into three forms, including supercoiled (SC), open circular (OC) and linear form (Linear). The low content of SC indicated that the DNA was damaged strongly. As present in Fig. 3, plasmid DNA was obviously damaged by the hydroxyl radical generated from the Fenton reaction (Lane 2), compared with plasmid DNA control (Lane 1). And the percentage of SC form in plasmid DNA decreased to 25.56% under the attack of free radicals (Fig. 4A). With the protection of APC, the oxidative injury of hydroxyl radical was alleviated (Lane 3–Lane 7 in Fig. 3), and the percentages of SC form in plasmid DNA were 28.79%, 35.78%, 46.21%, 58.69% and 72.19% (Fig. 4A) for different concentrations of APC (20–320 μg/mL), respectively. The results observed in Figs. 3 and 4A indicated that the oxidative damage of DNA caused by hydroxyl radical could be effectively inhibited with APC, which were concentration-dependent.

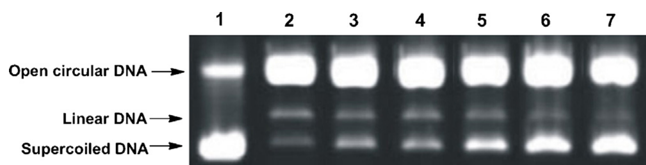

Fig. 3. Agarose gel electrophoretic patterns of plasmid pBR322 DNA. Lane 1, normal DAN control (only pBR322 DNA); Lane 2, DNA damage control (pBR322 DNA damaged by H_2O_2); and Lanes 3–7, DNA with the pretreatment of APC at different concentrations (25, 40, 80, 160 and 320 μg/mL).

V79 cell was used as a model to observe the protective effect of APC on cell injury caused by hydrogen peroxide, which was determined according the MTT test. Hydrogen peroxide (0.15 mM) caused a significant decrease in cell viability (37.86%, Fig. 4B). Pretreatment of V79 cells with APC inhibited the oxidative injury of cells, which was a dose-dependent manner. The cell viability increased to 43.25%, 52.69%, 66.79%, 73.69% and 87.96% for cells treated with 0.02, 0.04, 0.08, 0.16 and 0.32 mg/mL of APC, respectively (Fig. 4B).

4. Discussions

In the metabolism of most organisms, oxidation is an important process for energy supplication. Meanwhile, some uncontrolled or fulsome oxidations may also happen to generate the reactive oxygen species (ROS), which are toxic to human health (Liochev, 2013). ROS are generally unstable and highly reactive due to their single and unbalanced electrons, and can cause the oxidative injury of human body, which are involved in the occurrence of many chronic diseases such as diabetes, cardiovascular diseases and cancer (Sugamura, & Keaney, 2011). In present study, a novel antioxidant polysaccharide (APC) from *C. deodara* pine needles was observed to possess the remarkable antioxidant activity to scavenge ABTS, DPPH, superoxide and hydroxyl radicals (Fig. 2), which was stronger than BHA. The excellent antioxidant activity of APC might be attributed to its special characterization and composition. According to the previous studies, antioxidant activity of biological macromolecules may involve many mechanisms, such as prevention of chain initiation, decomposition of peroxides, free radical scavenging, reducing capacity and the binding of metal ion catalyst (Yang & Zhang, 2009; Leopoldini, Russo, & Toscano, 2011). It had been reported that the molecular weight of polysaccharides was an important parameter influencing antioxidant activity and the low molecular weight showed the high antioxidant activities (Wang et al., 2010). Previous studies also observed that the composition of polysaccharides had closely relationship with their biological

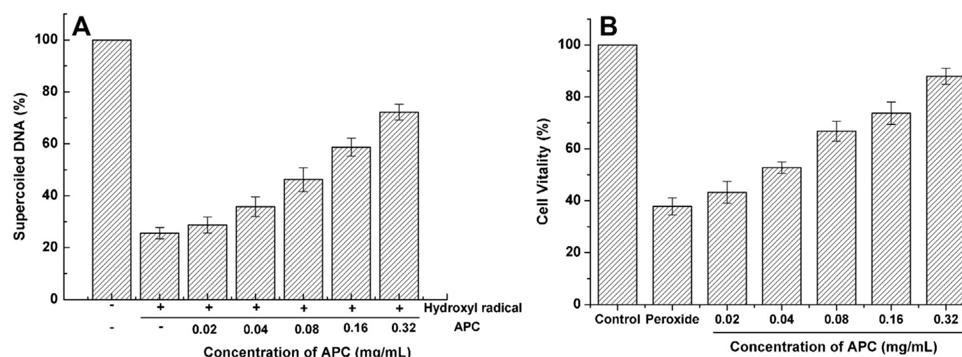

Fig. 4. Protective effect of APC on the oxidative injury of DNA and cells. (A) Quantification of supercoiled DNA in plasmid pBR322 DNA treated with various concentrations of APC; and (B) cell vitality of V79 cells treated with various concentrations of APC.

activity, especially for antioxidant activity (Chen, Xie, Nie, Li, & Wang, 2008; Yang & Zhang, 2009). In present study, analysis results indicated that APC was an acidic heteropolysaccharide with a molecular weight of 1.53×10^4 Da, and was composed of glucose, arabinose, mannose and xylose in a molar ratio of 37.53:1:4.32:0.93:0.91. Comparing with the previous report (Yang & Zhang, 2009), APC possessed the lower molecular weight and higher content of glucose, and these special characterization and composition might be propitious to the formation of hydrogen bond and enhance the hydrogen atom giving capability of APC to scavenge free radicals, which might improve its antioxidant activity. On the other hand, some relative studies had reported that the structure properties of polysaccharides had an important influence to their biological activities (Sun, 2011; Tu, 2012). According to the results of structural analysis, APC was observed to possess some branches composed by glucose and arabinose, and the backbone was mainly composed by glucose, mannose and xylose with $(1 \rightarrow 4)$ linked and a few of $(1\rightarrow)$ linked, $(1 \rightarrow 3)$ linked and $(1 \rightarrow 4,6)$ linked. It had been reported that the structure properties of polysaccharides, such as distribution of branch and the model of linked bond, might affect the complex interaction of monosaccharide in polysaccharides to improve its hydrogen atom giving capability, which played an important role for its antioxidant activity (Zhang, 1994; Zhang, Li, Xu, & Zeng, 2005; Chen, Roan, Lii, Huang, & Wang, 2011; Tu, 2012). Therefore, polysaccharides with different characterization and structure exhibited various biological activities. However, the exact mechanism of antioxidant activity of polysaccharides is still not fully understood.

Furthermore, our results demonstrated that APC had the remarkable antioxidant ability to protect the oxidative injury of DNA and cells. It had been reported that the ROS possessed the strong capacity to attack biological macromolecules, which could cause DNA damage, cell injury and many chronic diseases (Papaharalambus, & Griendling, 2007). As shown in Figs. 3 and 4A, APC exhibited the excellent property to inhibit the oxidative degradation of DNA, which might be attributed to its remarkable antioxidant capacity. As reported in previous study, iron ion could easily react with H_2O_2 to produce hydroxyl radicals to cause the oxidative degradation of DNA. So, free radical scavenger could inhibit the oxidative damage of DNA effectively (Aruoma, Halliwell, Gajewski, & Dizdaroglu, 1989). In present study, APC showed the remarkable hydroxyl radicals scavenging capacity (Fig. 2D), which might be the potential mechanism of APC to inhibit the oxidative degradation of DNA. In addition, ROS could also induce the peroxidation of lipid and protein on cell membrane and lead to the cell death, which was harmful to human health. Therefore, application of antioxidants might be an effective therapeutic strategy for the oxidative injury of cells (Castro, & Freeman, 2001). As shown in Fig. 4B, APC was observed to have an obvious capacity to inhibit the oxidative injury of V79 cells, which might be attribute to its intrinsic antioxidant activity, such as free radical scavenging activity (Fig. 2). It had been reported that the biological polysaccharides with antioxidant activity could protect cells against oxidative injury and/or alleviated the damage from ROS, which was due to their ability to donate hydrogen to free radicals and break the chain reaction of lipid oxidation at the first initiation step (Thetsrimuang, Khammuang, Chiablaem, Srisomsap, & Sarnthim, 2011; Wang et al., 2013). Previous studies also suggested that biological polysaccharides with high antioxidant activity might exhibit beneficial health functions and manifold pharmaceutical effects for human (Wu, Hu, Li, Huang, & Jiang, 2014; You, Yin, Zhang, & Jiang, 2014).

5. Conclusion

In present study, a novel antioxidant polysaccharide (APC) was isolated and characterized from pine needles of *C. deodara*, and showed a remarkable antioxidant activity to scavenge free radicals and to inhibit the oxidative injury of DNA and cells. Our results suggested that APC might be used as a potential antioxidant agent for preparing functional foods and nutraceuticals. Further studies are undergoing for the possible mechanism of antioxidant activity of APC and the toxicological evaluation for its safety usage.

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