Carbohydrate Polymers 144 (2016) 122–130

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

The antioxidant activities effect of neutral and acidic polysaccharides from *Epimedium acuminatum* Franch. on *Caenorhabditis elegans*

Zhou Xu, Shiling Feng, Shian Shen, Handong Wang, Ming Yuan, Jing Liu, Yan Huang, Chunbang Ding*

College of Life Science, Sichuan Agricultural University, Ya'an 625014, PR China

ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form 12 January 2016
Accepted 14 February 2016
Available online 18 February 2016

Keywords:
Acidic polysaccharide
Antioxidant activities
Caenorhabditis elegans
Epimedium acuminatum Franch
Neutral polysaccharide

ABSTRACT

A neutral polysaccharide (EAP-1N) and an acidic polysaccharide (EAP-2A) were purified from *Epimedium acuminatum* by DEAE-52 cellulose anion-exchange chromatography and gel-filtration chromatography. Their structures were characterized by chemical composition analysis, high-performance size exclusion chromatography (HPSEC), Fourier transform infrared spectrometry (FT-IR), and gas chromatography–mass spectrometry (GC–MS). Further, their antioxidant activities were investigated both *in vitro* and *in vivo*. Results showed that EAP-2A had higher uronic acid content and larger average molecular weight than EAP-1N. Compared with EAP-1N, EAP-2A exhibited significantly scavenging activities against free radical *in vitro*, as well as strongly stimulating effect on antioxidant enzyme activities (including superoxide dismutases (SOD), catalases (CAT), and glutathione peroxidases (GSH-PX)) and preferably inhibitory effect on lipid peroxidation and protein carboxyl in the mode of *Caenorhabditis elegans*.

1. Introduction

Reactive oxygen species (ROS), the generic term for chemically reactive molecules containing oxygen, are continuously produced as byproducts of normal mitochondrial electron transport and other metabolism processes (Blasiak, Hoser, Bialkowska-Warzecha, Pawlowska, & Skorski, 2015; Desouki, Kulawiec, Bansal, Das, & Singh, 2005). It is believed that low levels of ROS play a critical roles in maintaining homeostasis, delivering signal and regulating development. However, high concentrations of ROS are causing damage to proteins, lipids and nucleic acids (Burdon, 1995; Ng et al., 2014; Scherz-Shouval & Elazar, 2011). Furthermore, a large amount of *in vitro* and *in vivo* evidences suggest that high levels of ROS result in metabolic disorders such as inflammation, neurodegeneration, cancer and aging (Halliwell, 2006; Sabharwal & Schumacker, 2014; Shaw, Werstuck, & Chen, 2014).

Normally, enzymatic and non-enzymatic antioxidant defense systems maintain ROS under damage threshold in organism. Antioxidant enzyme system contains superoxide dismutases (SOD), catalases (CAT), and glutathione peroxidases (GSH-PX). Non-enzymatic antioxidant system consists of low-molecular weight compounds, such as ascorbic acid, ubiquinone, and uric acid (Sahiner & Cansin Sackesen, 2012; Sies, 1997). Moreover, natural products, vitamins, polyphenols and polysaccharides can potently scavenge ROS (Bułdak et al., 2014; Grünz et al., 2012; Hazewindus, Haenen, Weseler, & Bast, 2012). Particularly, polysaccharides isolated from traditional Chinese medicine show increasingly free radical scavenging activities and organism protecting property (Jiang et al., 2015; Shen et al., 2014; Wang, Wang, Su, & Zhang, 2014).

Epimedium acuminatum Franch., a perennial herb belongs to the family Berberidaceae, is widely distributed in China, Japan and Korean Peninsula (Wu, Lien, & Lien, 2003). In China, *Epimedium* has traditionally been used as tonic and aphrodisiac, as well as for the treatment of amnesia, osteoporosis, lumbago, arthritis, numbness and weakness of the limbs for thousands of years (Guo & Xiao, 2003; Him-Che, 1985). Accumulating evidences show that *Epimedium* polysaccharides exhibit excellent immune enhancement, anticancer and antioxidant activities *in vitro*, are one of the most important pharmacological active substances. (Cheng, Feng, Jia et al., 2013; Cheng, Feng, Shen et al., 2013; Guo et al., 2012). However, to our knowledge, a comparative study of the properties and antioxidant activities between neutral and acidic polysaccharides purified from *Epimedium* has not been assessed so far. Therefore, in the present study, a neutral and an acidic polysaccharides from *Epimedium* were obtained by anion-exchange and gel filtration

* Corresponding author.
E-mail address: dcb@sicau.edu.cn (C. Ding).

http://dx.doi.org/10.1016/j.carbpol.2016.02.041
0144-8617/

chromatography. Their physicochemical properties were characterized by chemical composition, Fourier transform infrared (FT-IR) spectroscopy, high-performance size exclusion chromatography (HPSEC), and gas chromatography–mass spectrometry (GC–MS). Moreover, *in vitro* antioxidant abilities and *in vivo* antioxidant abilities based on *Caenorhabditis elegans* model were also investigated.

2. Materials and methods

2.1. Materials and reagents

The aerial part of *E. acuminatum* was collected in September 2014 from Ya'an, Sichuan Province. Then, the fresh sample was washed, dried, and ground into powder with a high speed mill (FW177, Taisite Instrument Co., Ltd.), and stored in a desiccator at room temperature.

2,2-Diphenyl-1-picryl-hydrazyl (DPPH), phenazine methosulfate (PMS), dihydronicotineamide adenine dinucleotide (NADH), and nitrobluetetrazolium (NBT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) and penicillin–streptomycin were purchased from Gibco/BRL (Burlington, Canada). Ethanol, phenol, ferrous sulfate, sodium salicylate, yeast extract powder, agar, peptone, calcium chloride, cholesterol, sulphuric acid, glucose and coomassie brilliant blue G-250 were purchased from the Chengdu Kelong Chemical Factory (Chengdu, China). All chemicals were analytical grade.

2.2. Extraction and purification of polysaccharides

The dried *Epimedium* powder was defatted with petroleum ether (60–90 °C), and pretreated with 80% alcohol to remove pigment, oligosaccharides and some small molecule materials. The organic solvent was volatilized to obtain preparation sample (Wang, Liu, Huo, Zhao, Ren, & Wei, 2013; Ye & Jiang, 2011). Then, the crude *Epimedium* polysaccharides were extracted with hot distilled water from the residue and deproteinized by Sevage solution (chloroform: butyl alcohol, 4:1) according to a previously reported method (Cheng, Feng, Jia et al., 2013; Cheng, Feng, Shen et al., 2013).

Crude polysaccharides were redissolved in deionized water, filtered with 0.45-μm millipore filter, loaded on a DEAE-52 cellulose column (5.0 × 40 cm) and stepwise eluted with 0, 0.5 and 1.0 M NaCl at a rate of 3 mL/min (6 mL/tube). Total carbohydrate content of the eluate was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1951). The fraction eluted with distilled water (EAP-1) was collected, concentrated, dialyzed, dried and further purified through a sephadex G-100 column (2.0 cm × 50 cm) to obtain the neutral polysaccharide fraction EAP-1N. The same procedure as described above was implemented on the fraction eluted with 0.5 M NaCl (EAP-2), obtained the acidic polysaccharide fraction named EAP-2A.

2.3. Preliminary characterization of EAP-1N and EAP-2A

2.3.1. Chemical properties

Total carbohydrate content of polysaccharides was determined by the phenol–sulfuric acid colorimetric method, with glucose as the standard (Dubois et al., 1951). The protein content was quantified using the Bradford method, with bovine serum albumin as the standard (Wei, Li, & Tong, 1997). The uronic acid content was assessed by *m*-hydroxydiphenyl method, with D-galacturonic acid as the standard (Blumenkrantz & Asboe-Hansen, 1973). The average molecular weight of polysaccharides was estimated by high-performance size exclusion chromatography (HPSEC) method as described (Zeng, Zhang, & Jia, 2014), using a set of dextran standards (T-10, T-40, T-70, T100, T-200 and T-500) constructed molecular weight–distribution curve.

2.3.2. Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectrum of the polysaccharides was carried out using a FT-IR spectrophotometer (Shimadzu 8400s, Japan) in the frequency range of 4000–500 cm^{-1} *via* the potassium bromide (KBr) pressed-disc method (Parikh & Madamwar, 2006).

2.3.3. Monosaccharide composition

Monosaccharide composition of polysaccharides was performed by gas chromatography–mass spectrometry (GC–MS) as described previously (Shen et al., 2014). Briefly, polysaccharide sample was hydrolyzed with 3 mL of 3 M trifluoroacetic acid (TFA) at 100 °C for 8 h. Then, the hydrolysate was reduced by $NaBH_4$ (20 mg) at room temperature for 8 h, and acetylated by acetic anhydride (1 mL) and pyridine (1 mL) at 60 °C for 5 h. Finally, the acetyl derivative was dissolved in 2 mL chloroform and filtered through 0.22-μm millipore filter before analysis. The sample was analyzed by gas chromatography–mass spectrometry (GC–MS, QP2010, Shimadzu, Japan) equipped with a RTX-5 ms fused silica capillary column (0.25 μm × 0.25 mm × 30 m). The operation conditions of GC–MS were as follows: flow rate of the carrier gas (He) was 3 mL/min; the temperature of detector and inlet were 280 °C and 250 °C; the column temperature program was set changing from 120 °C (standing for 3 min) up to 210 °C (standing for 4 min) at a rate of 3 °C/min.

2.4. In vitro *antioxidant activity assay*

2.4.1. DPPH radical scavenging activity

The DPPH radical scavenging activities of polysaccharide fractions were determined according to a previous method (Shen et al., 2014) with slight modifications. Briefly, 1 mL polysaccharide sample (0–1.4 mg/mL) was added to 2 mL DPPH ethanol solution (0.2 mM), and then the absorbance was measured at 517 nm after incubated at 37 °C for 30 min. The DPPH radical scavenging effect was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(1 - \frac{A_S}{A_C}\right) \times 100$$

where A_S is the absorbance of DPPH solution with test sample and A_C is with replacement ethanol.

2.4.2. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activities of polysaccharide fractions were determined according to a previous method (Zhang, Lv, Jiang, Cheng, & Fan, 2015) with slight modifications. Briefly, 1 mL polysaccharide sample (0–1.4 mg/mL) was mixed with 0.5 mL $FeSO_4$ (1.5 mM), 0.35 mL H_2O_2 (6 mM) and 0.15 mL sodium salicylate (20 mM), and then the absorbance at 562 nm was measured after incubated at 37 °C for 1 h. The hydroxyl radical scavenging effect was calculated using the following formula:

$$\text{Hydroxyl radical scavenging effect (\%)} = \left(1 - \frac{A_S}{A_C}\right) \times 100$$

where A_S is the absorbance of the hydroxylated salicylate complex with test sample and A_C is with replacement distilled water.

2.4.3. Superoxide radical scavenging activity

The superoxide radical scavenging activities of polysaccharide fractions were determined according to a previous method (Robak & Gryglewski, 1988) with slight modifications. Briefly, 1 mL polysaccharide sample (0–1.4 mg/mL) was mixed with 3 mL Tris–HCl (16 mM, pH 8.0) containing 557 μM NADH, 108 μM NBT and 45 μM PMS, and then the absorbance at 560 nm was measured

after incubated at room temperature for 5 min. The superoxide radical scavenging effect was calculated using the following formula:

$$\text{Superoxide radical scavenging effect}(\%) = \left(1 - \frac{A_S}{A_C}\right) \times 100$$

where A_S is the absorbance of the reaction solutions with test sample and A_C is with replacement distilled water.

2.5. In vivo *biological activity assay*

2.5.1. C. elegans *strain and culture conditions*

Wild-type *C. elegans* strain (N2) and *Escherichia coli* OP50 strain were obtained from *Caenorhabditis* Genetics Center (CGC). The *C. elegans* were grown and maintained under standard laboratory conditions at 20 °C on nematode growth medium (NGM) agar plates fed with *E. coli* OP50. The synchronized populations were obtained by sodium hypochlorite treatment of gravid hermaphrodites.

2.5.2. *Lethality and growth assay*

The toxicity of polysaccharides in *C. elegans* was performed according to a previously described method (Zhang et al., 2013). Briefly, polysaccharides treatment was performed for 24 h from the stage of L4-larvae for lethality and growth assay. For lethality assay, *C. elegans* were judged to be dead if they failed to respond to stimulus using a platinum wire. Growth was evaluated by body length using an Olympus SZX16 stereo microscope (Olympus Corp., Tokyo, Japan) with Image-Pro Express software.

2.5.3. *Experimental design*

In order to evaluate the in *vivo* antioxidant effect of EAP-1N and EAP-2A, paraquat, an intracellular ROS generator was used to induce oxidative stress in *C. elegans* as described (Sonani, Singh, Kumar, Thakar, & Madamwar, 2014) with slightly modified. Briefly, Age-synchronized *C. elegans* were grown to L4 larva stage on NGM (containing 50 μM FUDR to block reproduction). Then, young adult *C. elegans* were transferred to fresh NGM plates containing 1 mg/mL polysaccharides and 20 mM paraquat (Sigma–Aldrich, St. Louis, MO) continue to train 24 h. Finally, the *C. elegans* were harvested, suspended by sucrose floatation and cleaned from bacteria with M9 buffer.

2.5.4. *Antioxidant enzyme activities*

The aforementioned *C. elegans* were suspended in cold homogenized buffer (0.01 M Tris–HCl, 0.0001 M EDTA-2Na, 0.01 M sucrose, 0.8% NaCl) and homogenized by Tissue Lyser (JY-250, Zhejiang, China). The mixture was centrifuged at 2500 r/min for 10 min at 4 °C and upper aqueous layer was transferred to a new EP tube for enzymatic assay. SOD, CAT and GSH-PX activities were measured using commercial chemical assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the methods described in the instructions. Protein content was determined by the bicinchoninic acid assay (BCA assay) with bovine serum albumin as standard (Smith et al., 1985).

2.5.5. *Lipid peroxidation and protein carbonyl assay*

The lipid peroxide level in the *C. elegans* was estimated by using the thiobarbituric acid (TBA) colorimetric method (Esterbauer & Cheeseman, 1989) to measuring the malondialdehyde (MDA) content. The absorbance of thiobarbituric acid reactive substance (TBARS) was measured at 532 nm and its concentration was calculated using the molar extinction coefficient $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Protein carbonyl contents were quantificated according to the 2,4-dinitrophenylhydrazone (2,4-DNPH) method as described by Uchida and Stadtman (1993) based on the formation of protein hydrazone by protein carboxyl reaction with 2,4-DNPH. Carbonyl content was calculated using the molar absorbance coefficient ($22{,}000\ M^{-1}\ cm^{-1}$) and expressed as nanomoles per milligram protein.

Table 1
Chemical characteristics and average molecular weight of EAP-1N and EAP-2A.

Sample	EAP-1N	EAP-2A
Carbohydrate (%)	90.95 ± 1.44	83.45 ± 2.08
Protein (%)	0.28 ± 0.05	2.66 ± 0.09
Uronic acid (%)	0.32 ± 0.07	9.46 ± 0.54
Molecular weight (kDa)	40.17	104.55

2.6. *Statistical analysis*

The data were acquired from three independent experiments and analyzed statistically by one-way analysis of variance (ANOVA). All results were expressed as mean ± standard deviation (S.D.)

3. Results and discussion

3.1. *Isolation and purification of polysaccharides*

The crude polysaccharides were extracted from the acrial parts of *E. acuminatum* and fractionated by DEAE-52 cellulose anion-exchange chromatography column. Two fractions (Fig. 1a), respectively named EAP-1 (distilled water) and EAP-2 (0.5 M NaCl), were collected, concentrated, dialyzed, lyophilized. Then, they were purified by gel filtration chromatography of Sephadex G-100, and EAP-1N and EAP-2A were obtained. As shown in Fig. 1b and c, both fractions were in a single and symmetrical peak, suggesting that they were homogeneous polysaccharide.

3.2. *Preliminary characterization of polysaccharides*

3.2.1. *Chemical compositions and molecular weight of EAP-1N and EAP-2A*

The carbohydrate content, protein content, uronic acid content and molecular weight of EAP-1N and EAP-2A are summarized in Table 1. Both polysaccharide fractions had quite high carbohydrate content and extremely low protein content. However, there was still a significant difference between EAP-1N and EAP-2A in chemical compositions. Especially, uronic acid content of EAP-1N and EAP-2A were 0.32 and 9.46% respectively, indicating that EAP-1N was a neutral polysaccharide while EAP-2A was an acidic polysaccharide. In addition, based on HPSEC results, the average molecular weights of EAP-2A (104.55 kDa) was higher than that of EAP-1N (40.17 kDa).

3.2.2. *FT-IR spectrum*

The FT-IR results indicated that two samples had the polysaccharides typical peaks within the range of 3600–3000, 3000–2800, 1400–1200 and 1200–700 cm^{-1}. As shown in Fig. 2, the strong and broad intense peak around 3400 cm^{-1} was attributed to O—H stretching vibration while the weak peak at 2926 cm^{-1} was assigned to C—H (Nep & Conway, 2011). The band near 1600 and 1400 cm^{-1} were arisen from C=O asymmetric stretching vibration and the absorption peak at 1251 cm^{-1} of EAP-2A was caused by O—H (COOH) variable angle vibration, which indicated that EAP-2A was acidic polysaccharide (Liu et al., 2012). These results were consistent with the fact that EAP-2A had relative high uronic acid content (Table 1). Furthermore, EAP-1N and EAP-2A exhibited stronger absorption peak at 1095 and 1074 cm^{-1}, which showed the monosaccharides of both polysaccharides might be existed as pyranoside (Tadayoni, Sheikh-Zeinoddin, & Soleimanian-Zad, 2015). Additionally, EAP-2A exhibited absorption at 881 cm^{-1},

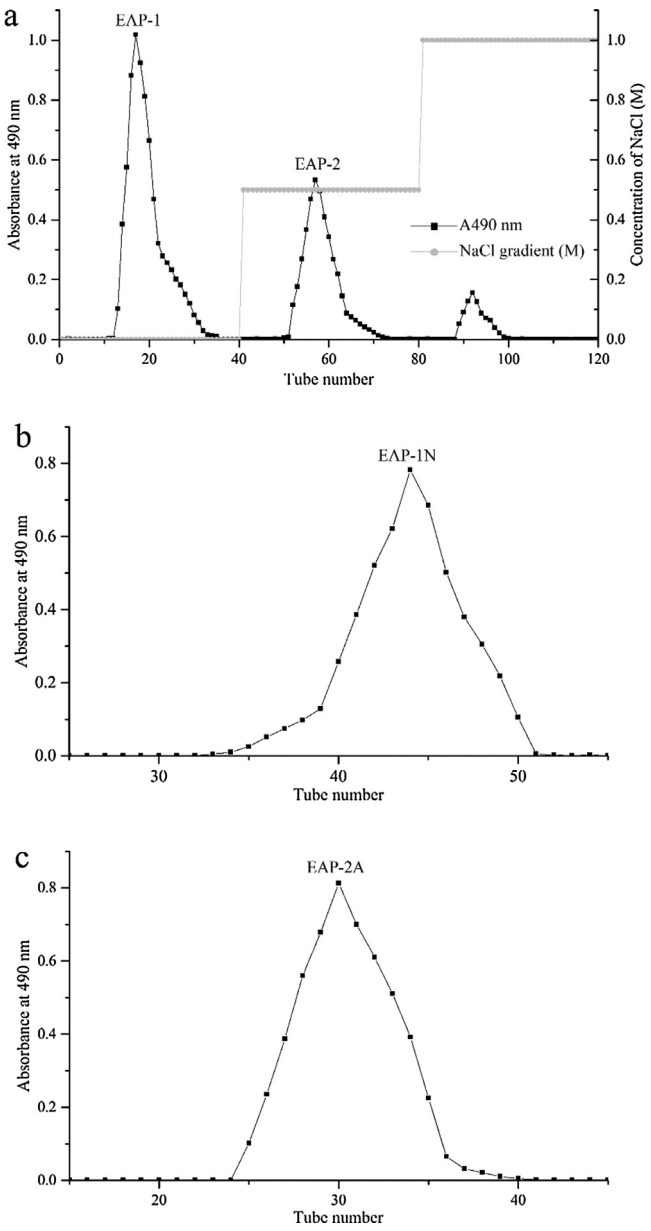

Fig. 1. (a) Elution profile of *Epimedium* polysaccharides on DEAE-52 chromatography column with gradient of NaCl solution (0, 0.5, and 1.0 M); (b) elution profile of EAP-1 on Sephadex G-100 gel chromatography column with distilled water; (c) elution profile of EAP-2 on Sephadex G-100 gel chromatography column with distilled water.

Table 2
Monosaccharide composition of EAP-1N and EAP-2A.

Samples	Monosaccharide content (molar ratio%)					
	Arabinose	Galactose	Glucose	Mannose	Rhamnose	Xylose
EAP-1N	12.24	1.29	26.05	2.59	–[a]	57.01
EAP-2A	9.09	5.68	22.26	6.09	1.67	55.20

[a] Not detected.

which suggested the presence of β-configuration (Barker, Bourne, Stacey, & Whiffen, 1954).

3.2.3. *Monosaccharide composition of EAP-1N and EAP-2A*

The monosaccharide composition results of EAP-1N and EAP-2A are summarized in Table 2. Neutral polysaccharide EAP-1N was composed of arabinose, galactose, glucose, mannose, and xylose, with the molar percentages of 12.24, 1.29, 26.87, 2.59, and 57.01%, respectively. Acidic polysaccharide EAP-2A was mainly composed of six monosaccharides, *i.e.*, arabinose, galactose, glucose, mannose, rhamnose and xylose, with the molar percentages of 9.09, 5.68, 22.26, 6.09, 1.67 and 55.20%, respectively. Xylose was the major monosaccharide of the two heteropolysaccharides fractions, which was distinctly different to the monosaccharide compositions of EAP40-1, EAP60-1 and EAP80-1 from *E. acuminatum* (Cheng, Feng, Jia et al., 2013; Cheng, Feng, Shen et al., 2013). This indicated EAP-1N and EAP-2A were two novel polysaccharide fractions. The difference of monosaccharide composition between this two polysaccharides and other *E. acuminatum* polysaccharides may be cause by many factors, including extraction technologies, purification methods and the sources of the *E. acuminatum*, *etc.*

3.3. In vitro *antioxidant activities of polysaccharide fractions*

3.3.1. *DPPH radical scavenging activity*

DPPH is a stable radical and appears purple in ethanol, when DPPH radical accepting a hydrogen (H) atom from scavenger

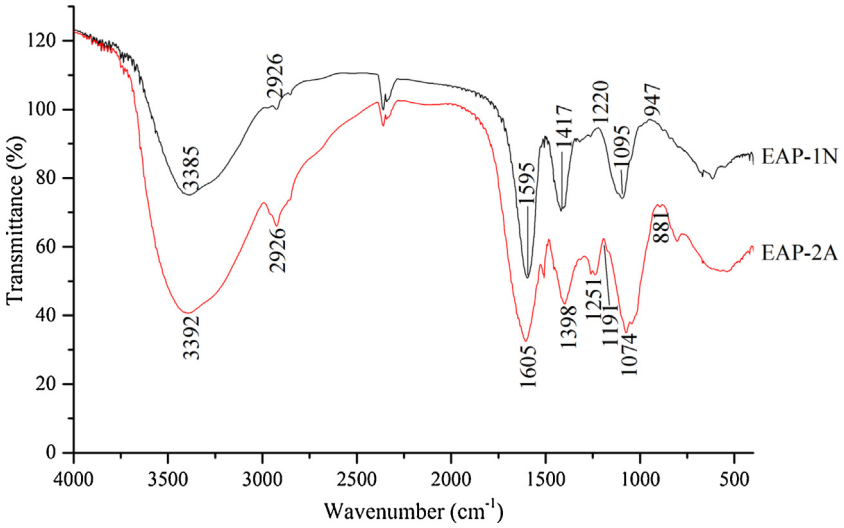

Fig. 2. FT-IR spectra of EAP-1N and EAP-2A.

molecules, the colour of its ethanol solution will changes from purple to yellow with concomitant decrease in absorbance at 517 nm (Mishra, Ojha, & Chaudhury, 2012). As shown in Fig. 3a, EAP-1N and EAP-2A displayed concentration-dependent DPPH radical scavenging effects. At 1.4 mg/mL, the scavenging activities for EAP-1N and EAP-2A were 30.69 and 73.31%, respectively. Previous reports showed that the antioxidant activity of polysaccharides correlated with their monosaccharide composition, uronic acid content, molecular weight and the type of glycosidic linkage (Seedevi et al., 2015; Zhang, Wang, Wang, Ma, Ye & Zeng, 2015). EAP-2A exhibited higher antioxidant activity than EAP-1N, which might be attribute to its higher uronic acid content and larger molecular weight of EAP-2A (Ma, Chen, Zhu & Wang, 2013).

3.3.2. *Hydroxyl radical scavenging activity*

Hydroxyl radical, one of the most harmful and reactive ROS, can easily across cell membranes and damage lipids, proteins and DNA. Hence, removing hydroxyl radical is important for protecting living system (Lipinski, 2011; Sahiner & Cansin Sackesen, 2012). As shown in Fig. 3b, the hydroxyl radical scavenging rates of EAP-1N and EAP-2A increased with sample concentration. At 1.4 mg/mL, the scavenging activities were 68.69 and 85.13% for EAP-1N and EAP-2A, respectively. These results indicated both EAP-1N and EAP-2A exhibited excellent hydroxyl radical scavenging abilities, which might be related to the number of active hydroxyl groups in polysaccharides (Guo et al., 2005).

3.3.3. *Superoxide radical scavenging activity*

Superoxide radical can easily react with other molecules and further form secondary radicals, such as the hydroxyl radical, peroxynitrate, hydrogen peroxide and singlet oxygen (Barroso et al., 2011; Wootton-Beard & Ryan, 2011). Therefore, the superoxide radicals scavenging activity is an important indicator of antioxidants. As shown in Fig. 3c, the results demonstrated that the polysaccharides exhibited obviously superoxide radicals scavenging activity in a concentration-dependent manner. At 1.4 mg/mL, the scavenging rates for EAP-1N and EAP-2A were 67.84 and 94.56%. The strong superoxide radical scavenging activity of EAP-2A might be partly due to its high content of carboxylic groups, which was in conformity with the result of uronic acid content (Guo et al., 2005; Yuan et al., 2015). But the specific mechanisms remained to be investigated further.

3.4. In vivo *biological activity analysis*

3.4.1. *Safety evaluation of polysaccharides on* C. elegans

Pretreatment with two polysaccharide fractions for 24 h, the safety property of them for L4 *C. elegans in vivo* were observed. The results showed that treatment with 0–1.0 mg/mL of polysaccharides did not influence their survival efficiency and development degree. However, once polysaccharides concentrations were higher than 1.0 mg/mL, *C. elegans* body length been affected (data not shown). Thus, 1.0 mg/mL was selected for the following *C. elegans* feeding tests.

3.4.2. *Antioxidant enzyme activities*

Reactive oxygen species (ROS), such as hydrogen peroxide, hydroperoxyl radical, superoxide anion and hydroxyl radical, are highly reactive molecules produced by cellular aerobic metabolism. *In vivo*, the excessive ROS may cause DNA mutation, protein and membrane lipid degradation, and ultimately lead to cancer, aging and many chronic diseases (Lipinski, 2011). Antioxidant enzymes, including SOD, CAT and GSH-PX, are crucial components of the antioxidant defense system in the body (Sahiner & Cansin Sackesen, 2012). SOD widely exists in all living cells and catalyzes superoxide radicals to hydrogen peroxide and oxygen. The main role of CAT and GSH-PX is to decompose hydrogen peroxide into a molecule of water and a molecule of oxygen (Kirkman, Rolfo, Ferraris, & Gaetani, 1999; Sahiner & Cansin Sackesen, 2012). Thus, collaborative work among these antioxidant enzymes in radical catabolic pathway can maintain ROS under a mild and appropriate levels.

Paraquat, a trigger generates ROS, can lead to oxidative stress. Therefore, paraquat is used as positive group, and the effects of EAP-1N and EAP-2A on the antioxidant defense system of *C. elegans* are investigated. As shown in Fig. 4a–c, compared to control group, treatment with paraquat significantly decreased the activities of SOD, CAT, and GSH-PX. However, compared to paraquat alone, the exogenous supplementation of polysaccharides (1 mg/mL) with paraquat remarkably increased activities of antioxidant enzymes. The activity of SOD, CAT, and GSH-PX increased by 11.7, 32.3 and 7.95% with supplementation of EAP-1N and by11.43, 52.20, 13.72% with supplementation of EAP-2A, respectively. The data suggested that both EAP-1N and EAP-2A had protective effect on antioxidant enzymes against oxidative stress. Meanwhile, previous researches have proved that polysaccharides from other sources can promote the activities of antioxidant enzymes (Feng et al., 2015; Gao et al., 2015; Guo & Qi, 2015). Gao et al. (2015) demonstrated that an acidic

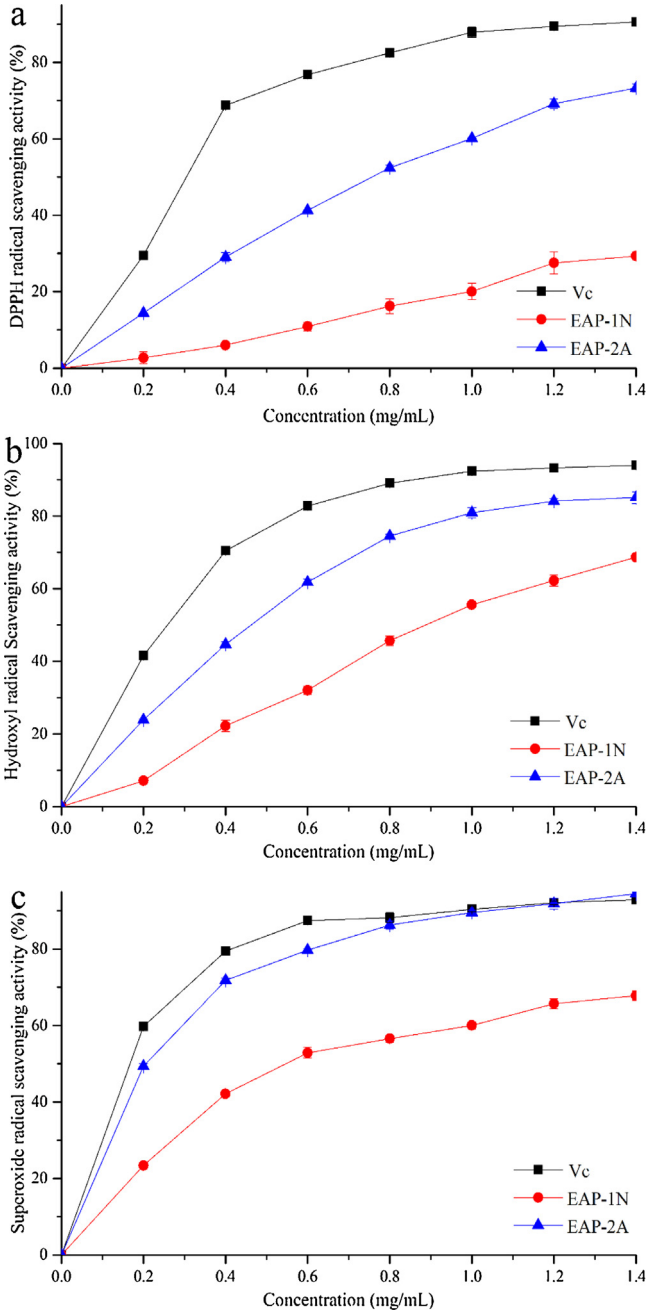

Fig. 3. (a) Scavenging effects of different concentration of EAP-1N, EAP-2A and ascorbic acid on DPPH radical, (b) hydroxyl radical and (c) superoxide radical.

polysaccharide purified from *Opuntia dillenii* Haw. fruits significantly increased the activities of SOD, CAT and GSH-PX in diabetic rats. Guo & Qi (2015) reported that *Cortex Eucommiae* polysaccharides observably ameliorated the activities of SOD, CAT and GSH-PX in the exhaustive exercise-induced oxidative stress mice. Moreover, our previous studies have also shown that polysaccharides from *Panax notoginseng* effectively upregulated the activities of antioxidant enzymes adapting to thermal stress resistance on *C. elegans* (Feng et al., 2015). In addition, our results manifested that EAP-2A exhibited stronger protective effect on antioxidant enzymes than EAP-1N, which might be partly due to the high uronic acid content and large molecular weight of EAP-2A. However, the exact mechanism of polysaccharides on the activities of antioxidant enzymes needs to be further investigated.

Values are given as mean ± S.D. for groups. Different letters indicate significantly different at $P < 0.05$.

3.4.3. Lipid peroxidation and protein carbonylation

Lipid peroxidation can commonly reflect the oxidation activity intensity *in vivo*. Fatty, especially polyunsaturated fatty acids, is easily attacked by ROS *via* non-enzymatic reactions, and thus yields an amount of byproducts that may be responsible for secondary damage to cells (Halliwell, 1990; Sultana, Perluigi, & Butterfield, 2013). Malondialdehyde (MDA), the end product of lipid peroxidation, can be considered as an indicator of fatty damage (Esterbauer & Cheeseman, 1989). Except for lipids, ROS can also react with proteins. At the cellular level, when proteins are exposed to ROS,

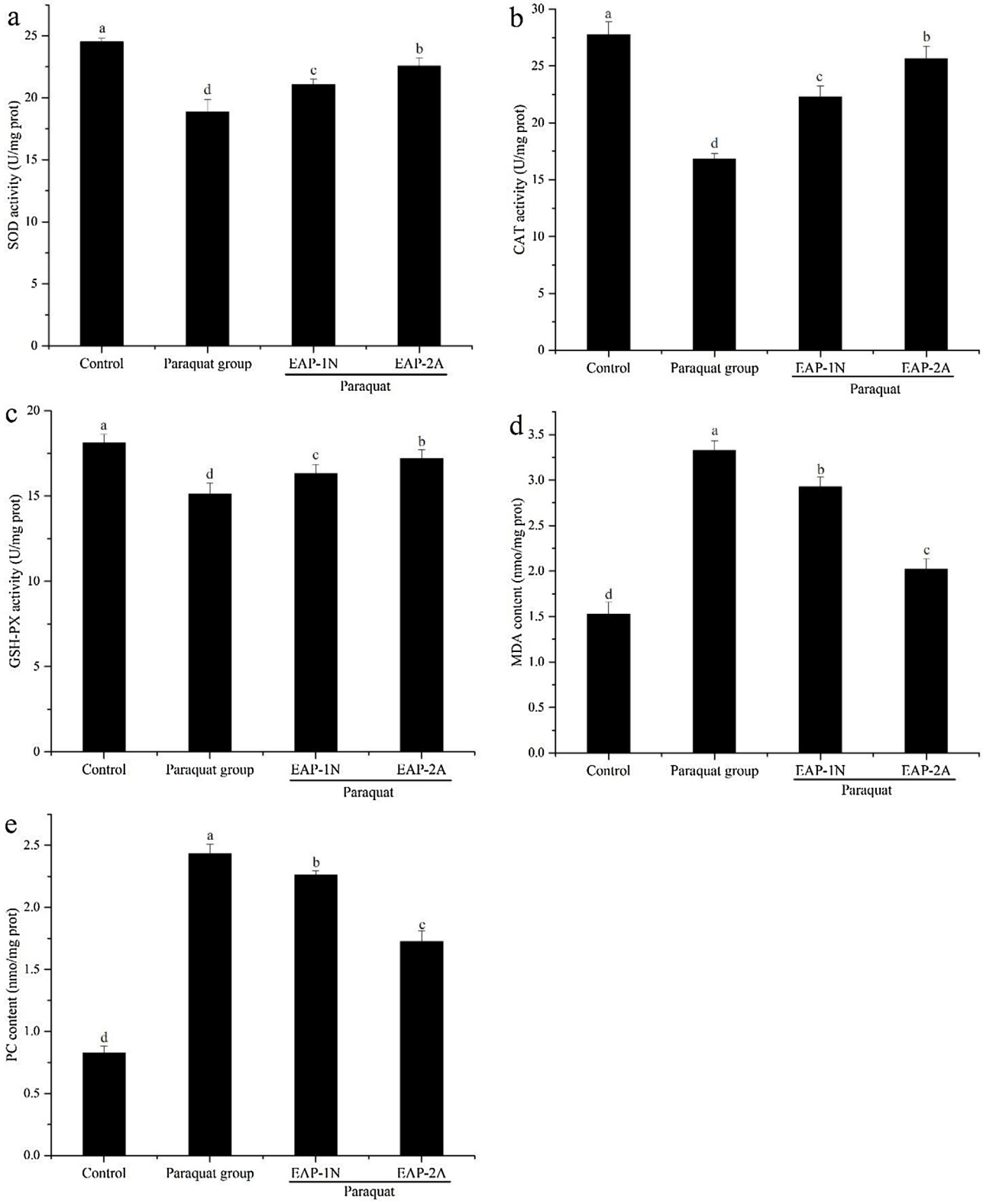

Fig. 4. (a) SOD activity, (b) CAT activity (c) GSH-PX activity, (d) malonaldialdehyde (MDA) content and (e) protein carbonyls (PC) content of *Caenorbabditis elegans* treated with paraquat (20 mM), paraquat (20 mM) + EAP-1N, and paraquat (20 mM) + EAP-2A.

irreversible formation of carbonyl groups (aldehydes and ketones) in amino acid lateral chain occurs, consequently, three-dimensional structure and biological function of protein are disrupted (Balabanlı & Balaban, 2015; Cabiscol, Tamarit, & Ros, 2010).

ROS are responsible for the lipids peroxidation and introduces carbonyl groups into proteins. Therefore, we quantitated the level of lipid peroxidation in terms of malonaldialdehyde (MDA) and protein carbonyls (PC) content. As shown in Fig. 4e and f, compared with paraquat group, the exogenous supplementation of

polysaccharides (1 mg/mL) along with paraquat could decrease the levels of MDA and PC *in vivo* by 12.08 and 7.16% with EAP-1N and by 39.32 and 29.12% with EAP-2A. The results shown that, the exogenous supplementation of polysaccharides could overcome oxidative injury of *C. elegans*. The decrease in the level of lipid peroxidation and protein peroxidation might be a result of the enhanced activity of endogenous antioxidant enzyme. Meanwhile, the experimental results further suggested that EAP-2A presented a stronger antioxidant capacity *in vivo* than EAP-1N, which revealed that the antioxidant activities of polysaccharides *in vivo* were mainly associated with their uronic acid content, molecular weight, monosaccharide composition (Wang, Shu et al., 2014). However, as was known to all, numerous factors including digestibility, bioavailability and metabolism of the compound also influence the antioxidant activities *in vivo* (Liu, Luo, Ye, Sun, Lu, & Zeng, 2010). Namely, the biological properties of polysaccharides were usually influenced by multi-factor, and the detail mechanism should be further investigated.

4. Conclusions

In this study, we purified a neutral polysaccharide (EAP-1N) and an acidic polysaccharide (EAP-2A) from *E. acuminatum*. The essential characteristic analytic results showed that EAP-1N and EAP-2A were quite different in their chemical composition, especially for uronic acid content, molecular weight, and monosaccharide composition. Compared with EAP-1N, EAP-2A exhibited stronger scavenging activities against free radical *in vitro*, as well as a strong stimulating effect on antioxidant enzyme activities (including SOD, CAT, and GSH-PX) and a preferable inhibitory effect on lipid peroxidation and protein carboxyl in the mode of *C. elegans in vivo*. The excellent antioxidant activities of EAP-2A might be correlated with its high uronic acid content and large molecular weight. These findings suggested that EAP-2A might have promising potential for application prospect in pharmacology and functional food. Further works on structure-activity relationship of EAP-2A are in progress.

References

Balabanlı, B., & Balaban, T. (2015). Investigation into the effects of boron on liver tissue protein carbonyl, MDA, and glutathione levels in endotoxemia. *Biological Trace Element Research*, *167*(2), 259–263.

Barker, S., Bourne, E., Stacey, M., & Whiffen, D. H. (1954). Infra-red spectra of carbohydrates. Part I: some derivatives of D-glucopyranose. *Journal of the Chemical Society*, *1*, 171–176.

Barroso, M. F., De-Los-Santos-Álvarez, N., Lobo-Castañón, M. J., Miranda-Ordieres, A. J., Delerue-Matos, C., Oliveira, M. B. P. P., et al. (2011). Electrocatalytic evaluation of DNA damage by superoxide radical for antioxidant capacity assessment. *Journal of Electroanalytical Chemistry*, *659*(1), 43–49.

Blasiak, J., Hoser, G., Bialkowska-Warzecha, J., Pawlowska, E., & Skorski, T. (2015). Reactive oxygen species and mitochondrial DNA damage and repair in BCR-ABL1 cells resistant to imatinib. *BioResearch Open Access*, *4*(1), 334–342.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*(2), 484–489.

Bułdak, Ł., Łabuzek, K., Bułdak, R. J., Kozłowski, M., Machnik, G., Liber, S., et al. (2014). Metformin affects macrophages' phenotype and improves the activity of glutathione peroxidase, superoxide dismutase, catalase and decreases malondialdehyde concentration in a partially AMPK-independent manner in LPS-stimulated human monocytes/macrophages. *Pharmacological Reports*, *66*(3), 418–429.

Burdon, R. H. (1995). Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radical Biology and Medicine*, *18*(4), 775–794.

Cabiscol, E., Tamarit, J., & Ros, J. (2010). Oxidative stress in bacteria and protein damage by reactive oxygen species. *International Microbiology*, *3*(1), 3–8.

Cheng, H., Feng, S., Jia, X., Li, Q., Zhou, Y., & Ding, C. (2013). Structural characterization and antioxidant activities of polysaccharides extracted from *Epimedium acuminatum*. *Carbohydrate Polymers*, *92*(1), 63–68.

Cheng, H., Feng, S., Shen, S., Zhang, L., Yang, R., Zhou, Y., et al. (2013). Extraction, antioxidant and antimicrobial activities of *Epimedium acuminatum* Franch. polysaccharide. *Carbohydrate Polymers*, *96*(1), 101–108.

Desouki, M. M., Kulawiec, M., Bansal, S., Das, G. C., & Singh, K. K. (2005). Cross talk between mitochondria and superoxide generating NADPH oxidase in breast and ovarian tumors. *Cancer Biology & Therapy*, *4*(12), 1367–1373.

Dubois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F. (1951). A colorimetric method for the determination of sugars. *Nature*, *168*, 167.

Esterbauer, H., & Cheeseman, K. H. (1989). Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods in Enzymology*, *186*, 407–421.

Feng, S., Cheng, H., Xu, Z., Shen, S., Yuan, M., Liu, J., et al. (2015). Thermal stress resistance and aging effects of *Panax notoginseng* polysaccharides on *Caenorhabditis elegans*. *International Journal of Biological Macromolecules*, *81*, 188–194.

Gao, J., Han, Y., Jin, Z., Xu, X., Zha, X., Chen, H., et al. (2015). Protective effect of polysaccharides from *Opuntia dillenii* Haw. fruits on streptozotocin-induced diabetic rats. *Carbohydrate Polymers*, *124*, 25–34.

Grünz, G., Haas, K., Soukup, S., Klingenspor, M., Kulling, S. E., Daniel, H., et al. (2012). Structural features and bioavailability of four flavonoids and their implications for lifespan-extending and antioxidant actions in *C. elegans*. *Mechanisms of Ageing and Development*, *133*(1), 1–10.

Guo, R., & Qi, B. (2015). Protective effect of polysaccharides from *Cortex Eucommiae* on exhaus-tive exercise-induced oxidative stress in mice. *Biomedical Research*, *26*(2), 375–379.

Guo, Z., Xing, R., Liu, S., Yu, H., Wang, P., Li, C., et al. (2005). The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan. *Bioorganic & Medicinal Chemistry Letters*, *15*(20), 4600–4603.

Guo, L., Wang, D., Hu, Y., Zhao, X., Wang, Y., Yang, S., et al. (2012). Adjuvanticity of compound polysaccharides on chickens against Newcastle disease and avian influenza vaccine. *International Journal of Biological Macromolecules*, *50*(3), 512–517.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research Communications*, *9*(1), 1–32.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now? *Journal of Neurochemistry*, *97*(6), 1634–1658.

Hazewindus, M., Haenen, G. R., Weseler, A. R., & Bast, A. (2012). The anti-inflammatory effect of lycopene complements the antioxidant action of ascorbic acid and α-tocopherol. *Food Chemistry*, *132*(2), 954–958.

Jiang, Y., Wang, L., Zhang, L., Wang, T., Zhou, Y., Ding, C., et al. (2015). Optimization of extraction and antioxidant activity of polysaccharide from *Salvia miltiorrhiza* Bunge residue. *International Journal of Biological Macromolecules*, *79*, 533–541.

Kirkman, H. N., Rolfo, M., Ferraris, A. M., & Gaetani, G. F. (1999). Mechanisms of protection of catalase by NADPH Kinetics and stoichiometry. *Journal of Biological Chemistry*, *274*(20), 13908–13914.

Lipinski, B. (2011). Hydroxyl radical and its scavengers in health and disease. *Oxidative Medicine and Cellular Longevity*, *2011*, 809696.

Liu, J., Luo, J., Ye, H., Sun, Y., Lu, Z., & Zeng, X. (2010). *In vitro* and *in vivo* antioxidant activity of exopolysaccharides from endophytic bacterium *Paenibacillus polymyxa* EJS-3. *Carbohydrate Polymers*, *82*(4), 1278–1283.

Liu, H., Fan, Y., Wang, W., Liu, N., Zhang, H., Zhu, Z., et al. (2012). Polysaccharides from *Lycium barbarum* leaves: isolation, characterization and splenocyte proliferation activity. *International Journal of Biological Macromolecules*, *51*(4), 417–422.

Ma, L., Chen, H., Zhu, W., & Wang, Z. (2013). Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom *Inonotus obliquus*. *Food Research International*, *50*(2), 633–640.

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. *Food Chemistry*, *130*(4), 1036–1043.

Nep, E. I., & Conway, B. R. (2011). Physicochemical characterization of grewia polysaccharide gum: effect of drying method. *Carbohydrate Polymers*, *84*(1), 446–453.

Ng, L. F., Gruber, J., Cheah, I. K., Goo, C. K., Cheong, W. F., Shui, G., et al. (2014). The mitochondria-targeted antioxidant MitoQ extends lifespan and improves healthspan of a transgenic *Caenorhabditis elegans* model of Alzheimer disease. *Free Radical Biology and Medicine*, *71*, 390–401.

Parikh, A., & Madamwar, D. (2006). Partial characterization of extracellular polysaccharides from cyanobacteria. *Bioresource Technology*, *97*(15), 1822–1827.

Robak, J., & Gryglewski, R. J. (1988). Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology*, *37*(5), 837–841.

Sabharwal, S. S., & Schumacker, P. T. (2014). Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel? *Nature Reviews Cancer*, *14*(11), 709–721.

Sahiner, U. M., & Cansin Sackesen, M. (2012). Oxidative stress and antioxidant defense. *The World Allergy Organization Journal*, *5*(1), 9–19.

Scherz-Shouval, R., & Elazar, Z. (2011). Regulation of autophagy by ROS: physiology and pathology. *Trends in Biochemical Sciences*, *36*(1), 30–38.

Seedevi, P., Moovendhan, M., Sudharsan, S., Vasanthkumar, S., Srinivasan, A., Vairamani, S., et al. (2015). Structural characterization and bioactivities of sulfated polysaccharide from *Monostroma oxyspermum*. *International Journal of Biological Macromolecules*, *72*, 1459–1465.

Shaw, P. X., Werstuck, G., & Chen, Y. (2014). Oxidative stress and aging diseases. *Oxidative Medicine and Cellular Longevity*, *2014*, 569146.

Shen, S., Cheng, H., Li, X., Li, T., Yuan, M., Zhou, Y., et al. (2014). Effects of extraction methods on antioxidant activities of polysaccharides from camellia seed cake. *European Food Research and Technology*, *238*(6), 1015–1021.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, *82*(2), 291–295.

Smith, P., Krohn, R. I., Hermanson, G., Mallia, A., Gartner, F., Provenzano, M., et al. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*, *150*(1), 76–85.

Sonani, R. R., Singh, N. K., Kumar, J., Thakar, D., & Madamwar, D. (2014). Concurrent purification and antioxidant activity of phycobiliproteins from *Lyngbya* sp. A09DM: an antioxidant and anti-aging potential of phycoerythrin in *Caenorhabditis elegans*. *Process Biochemistry*, *49*(10), 1757–1766.

Sultana, R., Perluigi, M., & Butterfield, D. A. (2013). Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. *Free Radical Biology and Medicine*, *62*, 157–169.

Tadayoni, M., Sheikh-Zeinoddin, M., & Soleimanian-Zad, S. (2015). Isolation of bioactive polysaccharide from acorn and evaluation of its functional properties. *International Journal of Biological Macromolecules*, *72*, 179–184.

Uchida, K., & Stadtman, E. (1993). Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra-and intermolecular cross-linking reaction. *Journal of Biological Chemistry*, *268*(9), 6388–6393.

Wang, Y., Liu, Y., Huo, J., Zhao, T., Ren, J., & Wei, X. (2013). Effect of different drying methods on chemical composition and bioactivity of tea polysaccharides. *International Journal of Biological Macromolecules*, *62*, 714–719.

Wang, Q., Shu, Z., Xu, B., Xing, N., Jiao, W., Yang, B., et al. (2014). Structural characterization and antioxidant activities of polysaccharides from *Citrus aurantium* L. *International Journal of Biological Macromolecules*, *67*, 112–123.

Wang, Z., Wang, C., Su, T., & Zhang, J. (2014). Antioxidant and immunological activities of polysaccharides from *Gentiana scabra* Bunge roots. *Carbohydrate Polymers*, *112*, 114–118.

Wei, Y., Li, K., & Tong, S. (1997). A linear regression method for the study of the Coomassie brilliant blue protein assay. *Talanta*, *44*(5), 923–930.

Wootton-Beard, P. C., & Ryan, L. (2011). Improving public health?: The role of antioxidant-rich fruit and vegetable beverages. *Food Research International*, *44*(10), 3135–3148.

Wu, H., Lien, E. J., & Lien, L. L. (2003). Chemical and pharmacological investigations of *Epimedium* species: a survey. *Progress in Drug Research*, *60*, 1–57.

Ye, C., & Jiang, C. (2011). Optimization of extraction process of crude polysaccharides from *Plantago asiatica* L. by response surface methodology. *Carbohydrate Polymers*, *84*(1), 495–502.

Yuan, M., Jia, X., Yang, Y., Ding, C., Du, L., Yuan, S., et al. (2015). Effect of light on structural properties and antioxidant activities of polysaccharides from soybean sprouts. *Process Biochemistry*, *50*(7), 1152–1157.

Zeng, W., Zhang, Z., & Jia, L. (2014). Antioxidant activity and characterization of antioxidant polysaccharides from pine needle (*Cedrus deodara*). *Carbohydrate Polymers*, *108*, 58–64.

Zhang, W., Lv, T., Li, M., Wu, Q., Yang, L., Liu, H., et al. (2013). Beneficial effects of wheat gluten hydrolysate to extend lifespan and induce stress resistance in nematode *Caenorhabditis elegans*. *PLoS One*, *8*(9), e74553.

Zhang, Z., Lv, G., Jiang, X., Cheng, J., & Fan, L. (2015). Extraction optimization and biological properties of a polysaccharide isolated from *Gleoestereum incarnatum*. *Carbohydrate Polymers*, *117*, 185–191.

Zhang, Z., Wang, F., Wang, M., Ma, L., Ye, H., & Zeng, X. (2015). A comparative study of the neutral and acidic polysaccharides from *Allium macrostemon* Bunge. *Carbohydrate Polymers*, *117*, 980–987.