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Research Article

Antioxidant Activity *in vitro* and *in vivo* of *Auricularia auricula* Polysaccharides through Different Extraction Processes

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Abstract: In this study, antioxidant effects *in vitro* and *in vivo* by the *Caenorhabditis elegans* model of *Auriculaia auricula* polysaccharides (AAP1, AAP2 and AAP3) were used to compare these three kinds of polysaccharide extraction processes (hot water extraction, enzyme extraction and alkali extraction). And the monosaccharide composition of these polysaccharides was determined by Gas Chromatography (GC) method. Results suggested that AAP2, which was mainly composed of arabinose, D-xylose, 2-Deoxy-D-arabino-hexose, mannose, glucose and galactose with the molar ratio of 1:0.44:0.33:1.67:1:0.17, possessed stronger antioxidant activity. It could scavenge free radicals, up-regulation of antioxidant enzyme (superoxide dismutase (SOD) by 91.25% and catalase (CAT) by 36.67%, compared with control). It could also reduce the level of Reactive Oxygen Species (ROS) in C. elegans under oxidative stress. In conclusion, enzyme extraction is better in preparing AAP.

Keywords: *Auriculaia auricula* polysaccharide, antioxidant activity, extraction processes

INTRODUCTION

With the improvement of people's living standards, people has paid increasing attention to health and aging-resistant. Ageing is a natural process in all living organisms. In recent years, it has been suggested that oxidative stress is a root cause of the ageing process (Golden and Melov, 2001). Oxidative damage will increase with age (Martin and Grotewiel, 2006). Reactive Oxygen Species (ROS) are naturally produced during metabolism and play an important role in maintaining homeostasis (Kang *et al*., 2015). A wide range of ROS, including singlet oxygen, superoxide anion radicals and hydrogen peroxide, will generate, when organisms expose to exogenous and endogenous factors, then resulting in homeostatic imbalance (Bonnefont-Rousselot *et al*., 2000; Jun *et al*., 2007). Increased ROS levels cause the damages of lipids, proteins and nucleic acids (Kim *et al*., 2009; Ziegler *et al*., 2011), leading to various physiological disabilities. And often result in metabolic disorders such as inflammation, aging, cancer and hypertension (Lee *et al*., 2013).

Nowadays some bioactive polysaccharides isolated from natural sources, which are able to be free radical scavenger in the prevention of oxidative damage in living organism (Pang *et al*., 2000; Tsiapali *et al*., 2001), have attracted much attention from researchers in the field of biochemistry and pharmacology (Yang and Zhang, 2009). In addition, many researches indicate that polysaccharides generally have a strong antioxidant action and can be employed as novel potential antioxidants (Hu *et al*., 2003; Jiang *et al*., 2005; Li *et al*., 2007).

Auriculaia auricula, an ear-shaped fungus, has been widely used both as food and as medicine in East Asia, especially in China and Korea (Luo *et al*., 2009; Nguyen *et al*., 2012; Yang *et al*., 2011). It is acknowledged to be a healthy food because of its high nutritional value. It contains a high content of carbohydrates, amino acids, minerals and many other nutritional ingredients (Fan *et al*., 2007; Zeng *et al*., 2012). The fruiting bodies of *A. auricula* are rich in polysaccharides. Recently, it has been found that polysaccharides from *A. auricula* possess a large variety of beneficial effects, including antioxidant activity (Fan *et al*., 2007), anticoagulant activity (Yoon *et al*., 2003), tendency to reduce blood sugar and anti-tumor properties (Ma *et al*., 2010). These properties can certainly improve the well-being of the consumers (Acharya *et al*., 2004; Emikpe *et al*., 2010). Therefore, increasing attention has been paid to its exploitation and utilization. Studies on its medicinal function, or as a healthy functional food, need to be done.

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In this study, three different kinds of polysaccharide from *Auriculaia auricula* were respectively obtained by alkali extraction, enzyme extraction and hot water extraction and deproteinized by Sevag method. GC was used to analyze monosaccharide composition of polysaccharides. The evaluation of their antioxidant activity *in vitro* was determined by their free radical scavenging capacity, metal chelating ability and reducing power. Their antioxidant activity *in vivo* was also investigated using *C. elegans* model, including the effect of *A. auricula* polysaccharides on *C. elegans*' lifespan, antioxidant stress ability, activity of superoxide dismutase and catalase.

MATERIALS AND METHODS

Materials: *Auriculaia auricula* was purchased from Hangzhou Hua Dan farming products Co., Ltd (Hangzhou, Zhejiang, China). The fruiting bodies of *A. auricula* were washed and oven dried at 70°C; were then ground to a particle diameter size: 200-300μm and stored in a desiccator at room temperature prior to use. 1,1-Diphenyl-2-Picryl-Hydrazyl radical (DPPH), ferrozine, ferricyanide, Trifluoroacetic Acid (TFA), Trichloroacetic Acid (TCA), salicylic acid, aquae hydrogenii dioxidi, paraquat, pyrogallol, ascorbic acid, pyridine, acetic anhydride, hydroxylamine hydrochloride were obtained from Hangzhou Mike Chemical Instrument Co., Ltd. *Caenorhabditis elegans* strains N2 (wild-type) and *Escherichia coli* strain OP50 were obtained from Zhejiang University. Total Superoxide Dismutase (T-SOD) assay kit, catalase (CAT) assay kit and reactive oxygen species (ROS) assay kit were bought from Nanjing Jiancheng Bioengineering Institute. D-(-)-Arabinose, D-xylose, 2-Deoxy-D-arabino-hexose, D-Mannose, Glucose, N-acetyl-D-(+)-glucosamine were of analytical grade and bought from Sigma-Aldrich Chemical Co. (St. Louis, USA).

Extraction methods:

Hot water extraction: *Auriculaia auricula* powder (20 g) was extracted with 1 L of distilled water at 80°C for 4h. Then the samples were clarified by centrifugation at 8000 rpm for 10 min to remove residue.

Enzyme extraction: *Auriculaia auricula* powder (10 g) was homogenized in 500 mL of pH4.5, 0.05mol/L citric acid sodium citrate buffer solution, added cellulase 2% (w/w) for 90 min at 50°C. After the reaction, adjust the pH of homogenate to 7.0. Subsequently, the reaction was continued for 2 h, but the temperate of reaction was upped to 85°C. Then the samples were clarified by centrifugation at 8000 rpm for 10 min to remove residue.

Alkali extraction: *Auriculaia auricula* powder (3.5 g) was homogenized in 560 mL of 1% (w/v) sodium hydroxide solution and stirred for 3h at 80°C. After the reaction, using hydrochloric acid to adjust the pH of homogenate to 7.0 and then the samples were clarified by centrifugation at 8000 rpm for 10 min to remove residue.

Alcohol precipitation: The supernatants were combined, then precipitated with 95% ethanol (1:4, v/v), stored at 4°C for 6 h and then centrifuged at 8500 rpm for 10 min. The precipitate was re-dissolved in distilled water. The samples were stored at -20°C for the further experiments.

Deproteinization: The crude polysaccharides were put into blue cap bottle and treated with 1/5 volumn of sample of Sevag reagent (butyl alcohol: chloroform = 1:4, v/v) to remove proteins (Huang *et al*., 2010), repeated this treatment until there was no absorption under 260 nm on UV-Vis spectrophotometer. Samples were stored at -20°C after freeze drying.

Monosaccharide composition analysis: 10 mg sample was hydrolyzed with 2 mL of 2 M TFA at 110°C for 3 h. The excess acid was removed by vacuum evaporation with methyl alcohol (MeOH) after the hydrolysis. The hydrolyzed products were mixed with pyridine (0.5 mL) and 0.1 mL of 100 mg/mL hydroxylamine hydrochloride at 90°C for 30 min. Then cooling to room temperature, the reaction solution was acetylated with 0.6 mL of acetic anhydride at 90°C for 30 min (Zeng *et al*., 2012), the standard monosaccharide was derived with same method. The alditol acetates were analyzed by GC using a Shimadzu GC-2014C instrument equipped with an SG-54 capillary column (30 m×0.32 mm×0.25μm) and a flame ionization detector (FID). The applied temperature program was as follows: oven temperature was initially set at 120°C, then was increased to 240°C at a rate of 10°C/min and later held at 240°C for 6.5 min. The heater temperature of injector was 250°C, while detector was kept at 280°C. Nitrogen was used as the carrier gas.

Antioxidant activities of AAP *in vitro*:

DPPH radical-scavenging activity: The DPPH free radical scavenging activity of *Auriculaia auricula* polysaccharide was investigated according to the previous method with a modification (Zeng *et al*., 2012; Liang *et al*., 2011). DPPH solution (0.1 Mm, in ethanol) was prepared and used fresh on the day of each test. 2.0 mL sample (0.125–2 mg/mL) was mixed with 2.0 mL DPPH solution. The mixture was allowed to stand for 30 min at room temperature in the dark and absorbance was monitored at 517 nm. The antioxidant was calculated with the following equation:

$$\text{Scavenging effect (\%)} = (1-\frac{A_1-A_2}{A_3})\times 100\%$$

where, A_1 is the absorbance of test sample (DPPH solution with sample or positive control), A_2 is the absorbance of blank (sample with no DPPH) and A_3 is the absorbance of control(DPPH solution with no sample).

Superoxide radicals scavenging ability assay: Superoxide radicals scavenging activity was measured according to the previous method with some modification (Marklund and Marklund, 1974). The superoxide radicals generating system was based on the autoxidation of pyrogallol reaction. 4.0 mL sample (0.125-2 mg/mL) was mixed with 5.0 mL of 50 mM Tris-HCl buffer (pH 8.0). After incubating for 20 min at 25°C, a 1.0 mL of 3 mM pyrogallol which had been preheated was added to the mixture and incubated for 5min at 25°C. Then 1.0 mL of 10mM HCl was added to terminate the reaction and absorbance was monitored at 320 nm. Ascorbic acid was used as positive controls. The antioxidant was calculated with the following equation:

$$\text{Scavenging effect (\%)} = (1-\frac{A_1-A_2}{A_3})\times 100\%$$

where, A_1 is the absorbance of test sample (reaction solution with sample or positive control), A_2 is the absorbance of blank (sample with no pyrogallol) and A_3 is the absorbance of control(reaction solution with no sample).

Hydroxyl radicals scavenging activity: Hydroxyl radicals scavenging activity was measured according to the previous method with modification (Yang *et al*., 2011; Zhang *et al*., 2010). The hydroxyl radicals generating system was based on the Fenton reaction, so the reaction mixture included 1 mL sample (0.125-2 mg/mL), 2 mL of 1.8 mM $FeSO_4$, 1.5 mL of 1.8 mM salicylic acid, 0.1 mL of 0.03% H_2O_2. After incubating for 30 min at 37°C, absorbance was monitored at 510 nm. Ascorbic acid was used as positive controls. The antioxidant was calculated with the following equation:

$$\text{Scavenging effect (\%)} = (1-\frac{A_1-A_2}{A_3})\times 100\%$$

where, A_1 is the absorbance of test sample (reaction solution with sample or positive control), A_2 is the absorbance of blank (sample with no H_2O_2) and A_3 is the absorbance of control (reaction solution with no sample).

Metal chelating ability: 1.0 mL sample (0.125-2 mg/mL) was mixed with 0.1ml of 2.0 mM $FeCl_2$, 0.2 mL of 5.0 mM ferrozine, 3.7 mL distilled water. The mixture was allowed to stand for 10 min at room temperature. And then absorbance was monitored at 562 nm. EDTA was used as positive controls. The antioxidant was calculated with the following equation:

$$\text{Metal chelating ability\%} = (1-\frac{A_1-A_2}{A_3})\times 100\ \%$$

where, A_1 is the absorbance of test sample (reaction solution with sample or positive control), A_2 is the absorbance of blank (sample with no $FeCl_2$) and A_3 is the absorbance of control(reaction solution with no sample).

Reducing power assay: Reducing power was measured according to the method of Qi *et al.* (2005). 0.5 mL sample (0.125–2 mg/mL) was mixed with 1.25 mL of 0.2 M PBS buffer (pH 6.6), 1.25 mL of potassium ferricyanide and incubated for 20 min at 50°C. Then cooling quickly, 1.25 mL of TCA (10%, w/w) to end the reaction. Next, a 1.25 mL reaction solution was mixed with 1.25 mL of distilled water, 0.25 mL of $FeCl_3$ (0.1%, w/w) and standed for 10 min. Absorbance was monitored at 710 nm. Ascorbic acid was used as positive controls.

Antioxidant activities of AAP *in vivo:*

***C. elegans* culture:** The antioxidant effect *in vivo* of *Auriculaia auricula* polysaccharide was examined by a model animal, the N2 wild type *C. elegans*. Strains were cultured and maintained at 20°C on petri dishes containing nematode growth medium (NGM) seeded with a live *E.coli* strain OP50 as the food source as previously described (Brenner, 1974; Grünz *et al*., 2012; Kim *et al*., 2008). Synchronization of worms was achieved by preparing eggs from gravid adults using a solution containing 1.5% NaOCl and 1.5 M NaOH. Eggs were washed with M9 buffer and then allowed to hatch overnight on NGM agar plates without bacteria.

Lifespan assay: Lifespan analysis was carried out at 20°C on day 1 adult animals by placing larvae worms at L4 stage 1 day before starting lifespan assay. This transfer day was designated as Day 0. And 1 mg/mL *A. auricula* polysaccharide and 12.5 mg/mL of 5-FU were added to NGM. 5-FU was added to prevent adults from generating newborn progenies (Vayndorf *et al*., 2013). Worms were transferred to fresh NGM plates using a platinum pick wire every other day and the surviving worms were counted at the same time. The survival ratio was calculated from the percentage of living worms out of the total number of worms, including living and dead animals.

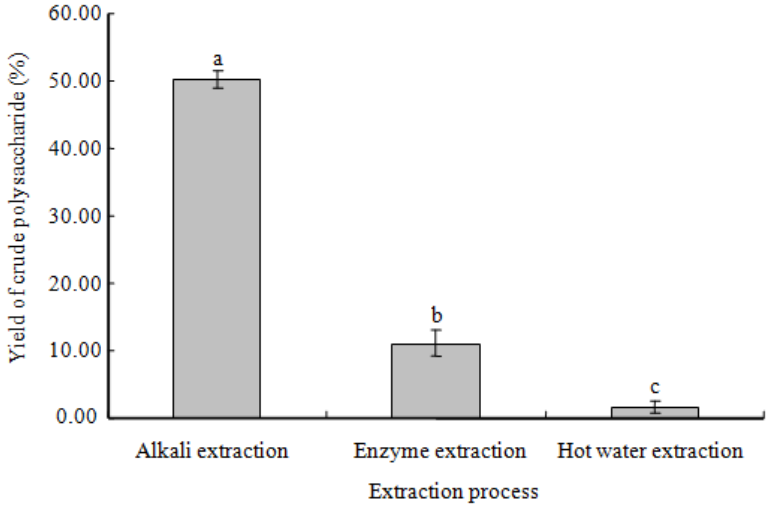


Fig. 1: Effect of extraction process on yield of crude polysaccharide. Bars marked without the same letters (a-c) differ significantly (P<0.05)

Oxidative stress resistance: Worms treated with *A. auricula* polysaccharide, ascorbic acid or M9 buffer on Day 5. After the treatment for 2 days, the worms were washed three times with M9 buffer and then exposed to 83.33 mg/mL paraquat in 96-well plates at 20°C. Surviving worms were counted every once in a while by prodding with pipette tips.

To examine resistance to H_2O_2, nematode were pretreated with *A. auricula* polysaccharides, ascorbic acid or M9 buffer for 2 days from Day 5 and subsequently exposed to 0.1 M H_2O_2 in 96-well plates. We scored the 5 h survival of N2 animals.

Measurement of SOD/catalase activity: Worms treated with 1.0 mg/mL *A. auricula* polysaccharide, 1.0 mg/mL ascorbic acid or M9 buffer on Day 5. After the treatment for 2 days, the worms were washed three times with M9 buffer and diluted to 1% (v/v). Then the activity of SOD or CAT was detected based on instruction of T-SOD assay kit or CAT assay kit.

Detection of ROS: On Day 5, treatment of N2 animals with 1.0 mg/mL *A. auricula* polysaccharide, 1.0 mg/mL ascorbic acid or M9 buffer was started and proceeded for 2 days as described aforementioned and then washed extensively. The worms were incubated with 83.33mg/mL paraquat for 3 h and then washed three times with M9 buffer. Subsequently, the worms were transferred to 2 mL of solution that contained 15μL of 0.1 mM H2DCF-DA, 185μL of 0.1 M PBS buffer (pH 7.2) and incubated for 30 min at 20°C. Shimadzu RF5301 fluorescence spectrophotometer was used to determine the fluorescence of DCF (excitation at 485 nm and emission at 525 nm) (Liu *et al*., 2001; Wang *et al*., 2007). The relative fluorescence could be figured out comparing with control. The statistical significance of differences between the control and treated groups were determined by SPSS.

Statistical analysis: Data are expressed as the mean ± S.D. Duncan's multiple range tests was used to determine the difference among AAPs and control groups with the software SPSS 19.0. Significant differences were considered as $p<0.05$

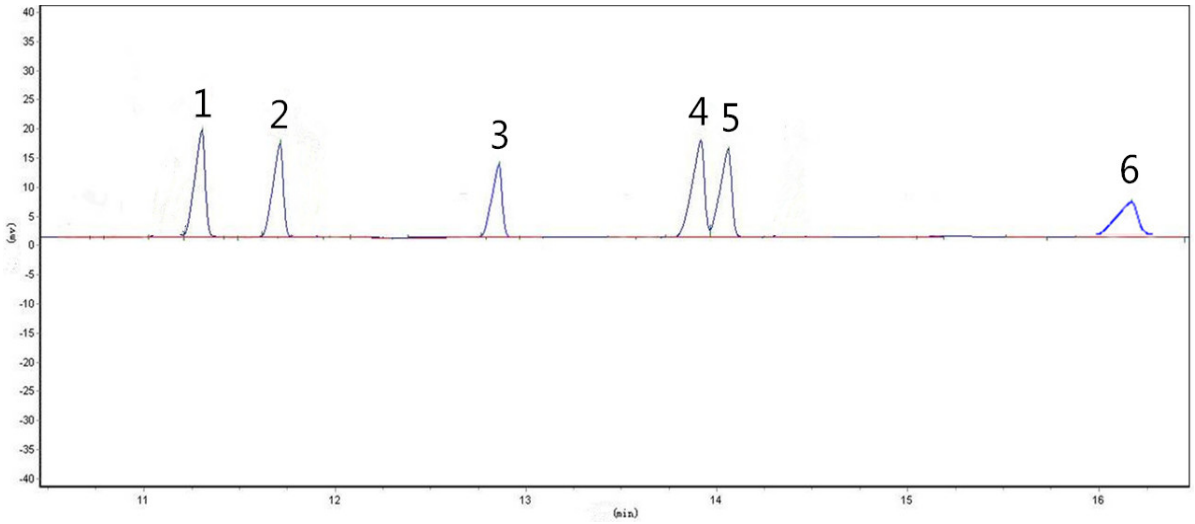
RESULTS AND DISCUSSION

Yield of crude polysaccharide: In Fig. 1, different extraction processes have different yield of crude polysaccharide. Among them, alkali extraction had the highest yield of crude polysaccharide, 50.35±1.25%. And the second was extraction with cellulose, 11.12±1.88%. Using hot water to extract crude polysaccharide had the lowest yield, 1.61±0.93%.

Monosaccharide composition analysis: In Fig. 2, the GC spectra profile of the AAP presented six peaks with six tention time of 11.225 min, 11.790 min, 12.708 min, 13.867 min, 13.983 min and 16.207 min. It was concluded that the AAP was a heteropolysaccharide and consisted of D-arabinose, D-xylose, 2-deoxy-D-arabino-hexose, D-mannose, glucose, N-acetyl-D-glucosamine in different molar ratio (Table 1).

Antioxidant activities of AAP *in vitro*:

DPPH radical-scavenging activity: DPPH is one of the compounds that possessed a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavenger (Song *et al*., 2010). On interacting with DPPH, antioxidants transfer either an electron or a hydrogen atom to DPPH, thus neutralizing its free radical



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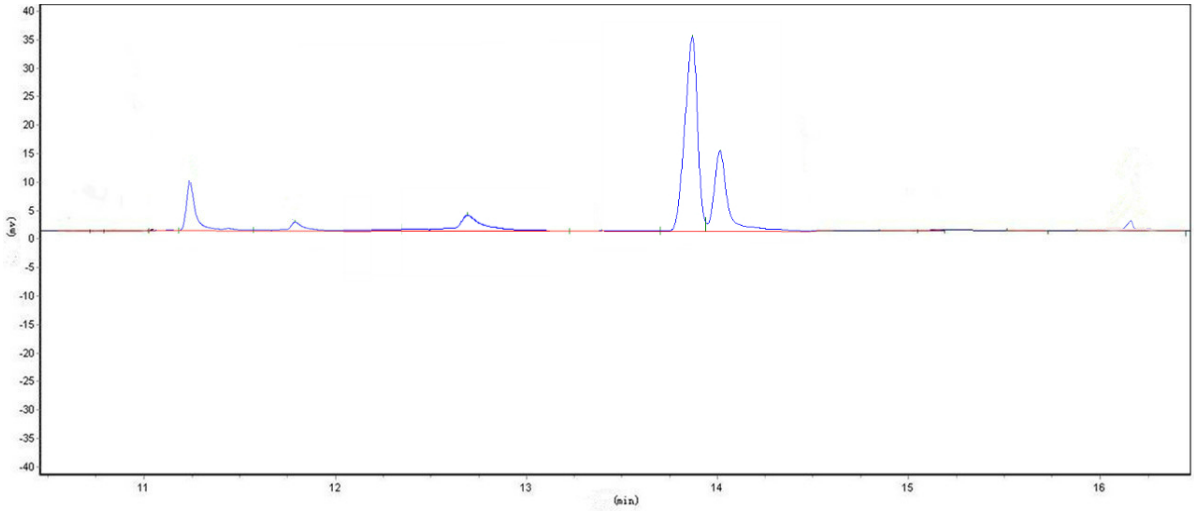

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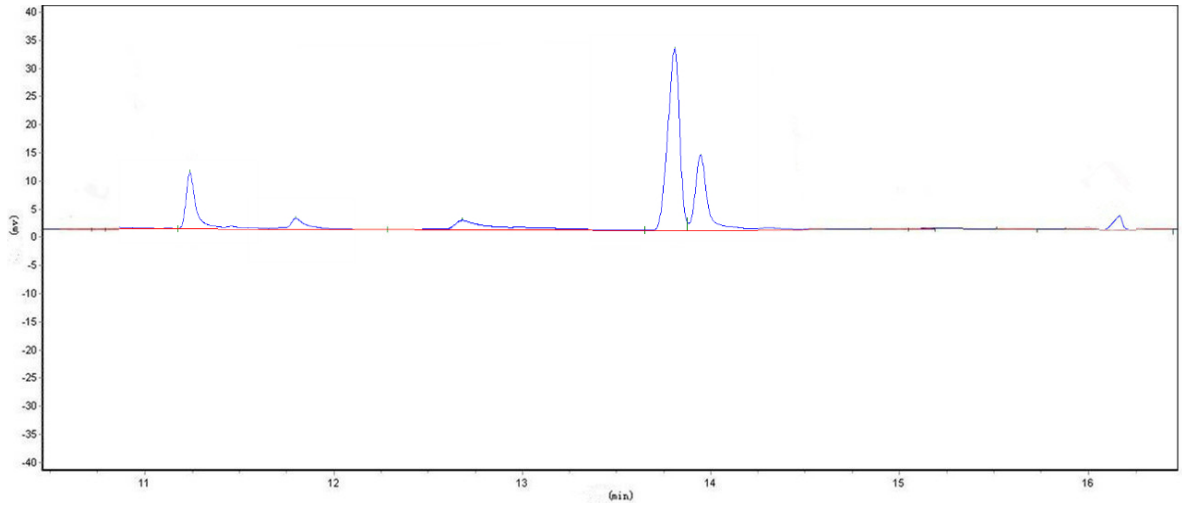

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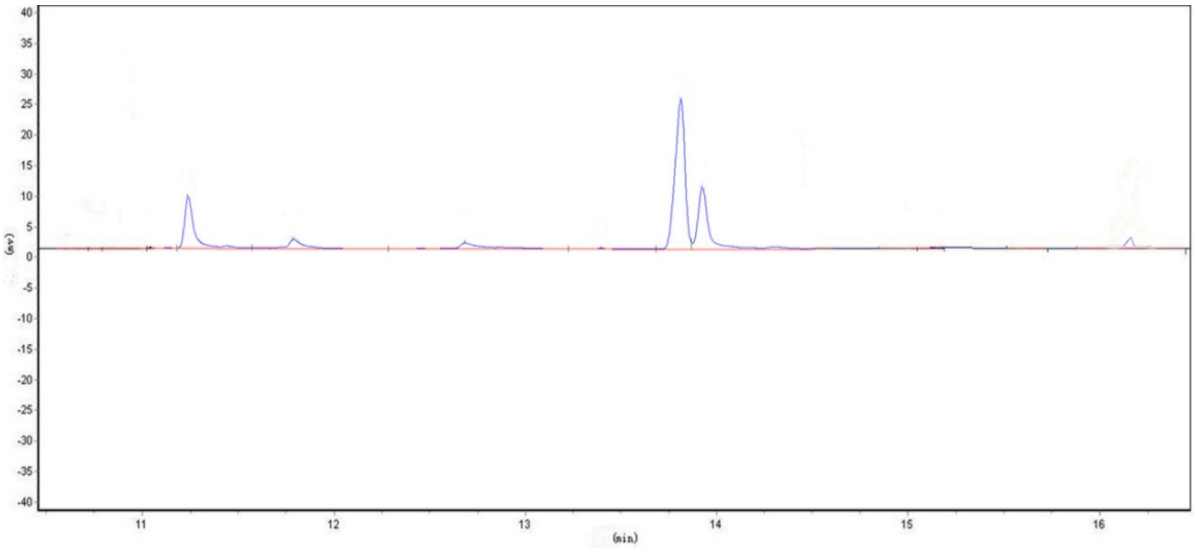
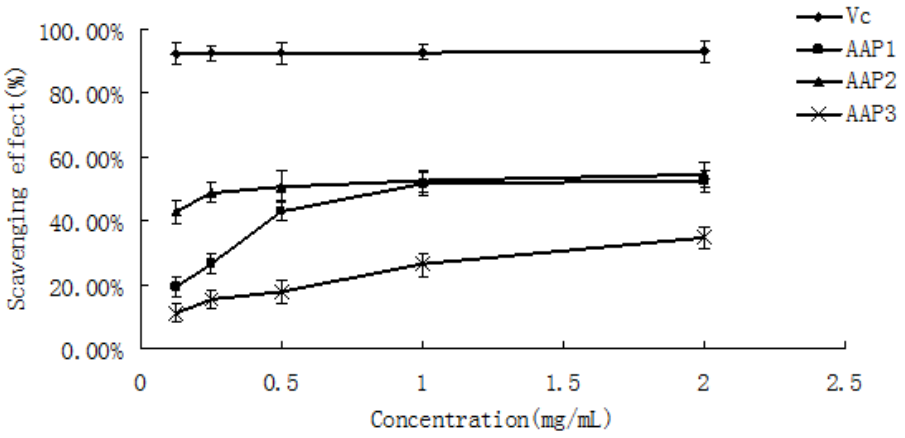


(d)

Fig. 2: GC spectrum (a, standard monosaccharide mixture (1: D-(-)-Arabinose; 2: D-xylose; 3: 2-Deoxy-D-arabino-hexose; 4: D-Mannose; 5: Glucose; 6: N-acetyl-D-(+)-glucosamine); b, AAP1; c, AAP2; d, AAP3)



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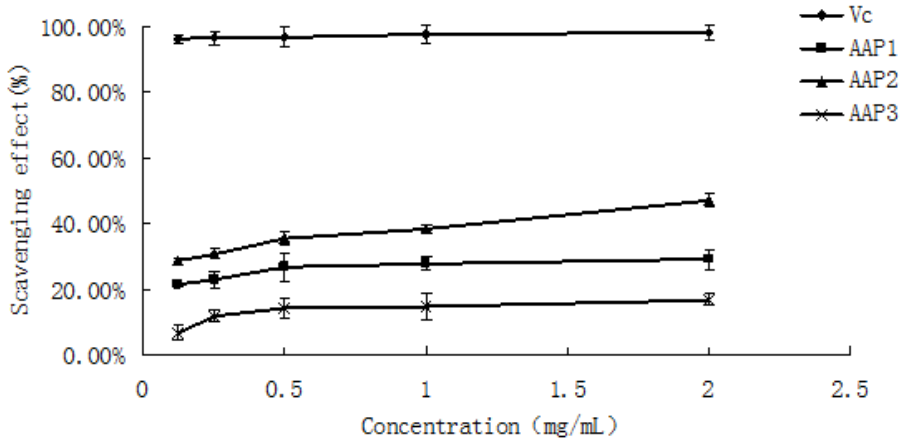

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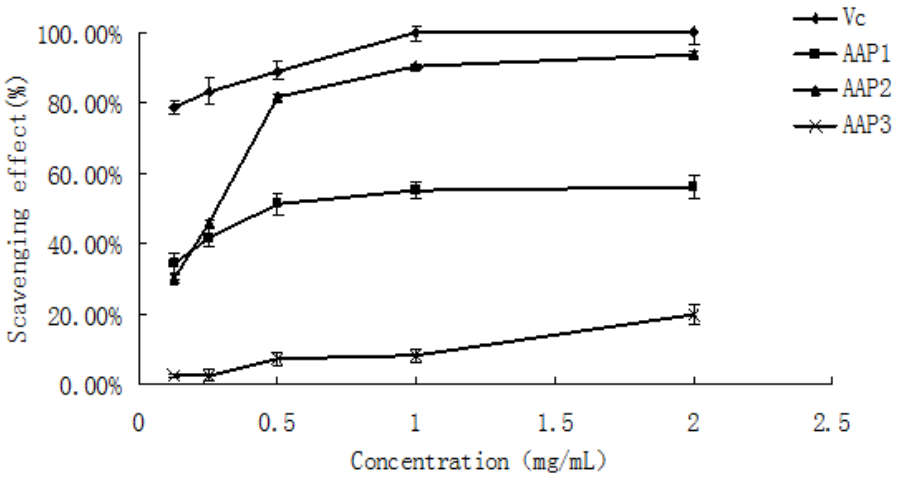

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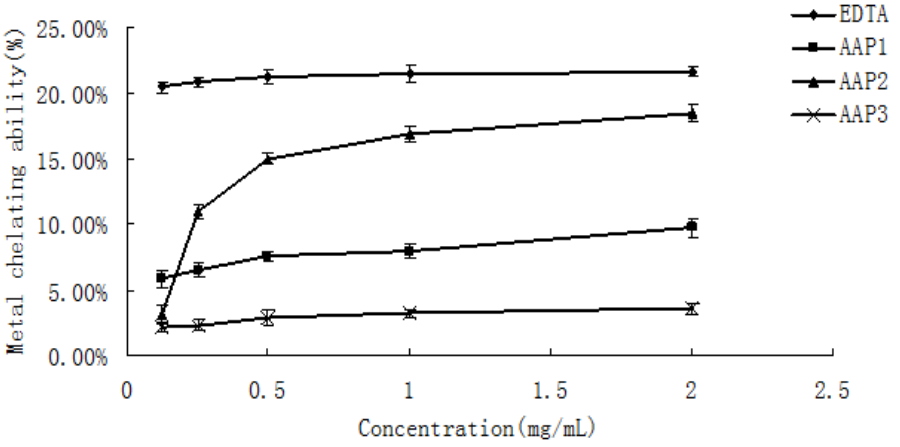

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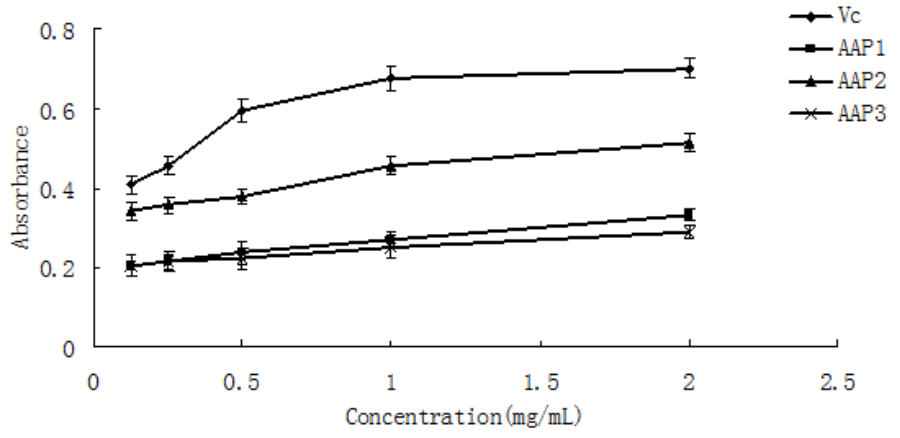


(e)

Fig. 3: Radical-scavenging activity (a, DPPH; b, superoxide radical; c, hydroxyl radical), metal chelating ability (d) and reducing power (e) of AAP

character (Naik *et al*., 2003). In Fig. 3a, at the concentration from 0.125 mg/mL to 2 mg/mL, the DPPH radical scavenging activity increase with the polysaccharide concentration, this increase became less

Table 1: Monosaccharide compositions of polysaccharides isolated from *A. auricular*

	Molar ratios					
Polysaccharide	D-Ara	D-Xyl	2-DG	D-Man	Glu	D-GlcNAc
AAP1	0.89	0.39	0.39	1.87	1	0.22
AAP2	0.88	0.36	0.45	1.76	1	0.11
AAP3	1.00	0.44	0.33	1.67	1	0.17

obvious when the concentration exceeded 0.5 mg/mL, whereas the overall DPPH radical scavenging activity was not as strong as ascorbic acid.

Superoxide radical-scavenging activity: The superoxide radical is a toxic species can be generated by numerous biological and photochemical reactions. In addition to directly attacking important biological molecules, superoxide radical may also decompose to form singlet oxygen and hydroxyl radicals, which may increase local oxidative stress and initiate cellular damage or lipid peroxidation and pathological incidents (Chen *et al*., 2012; Liu *et al*., 2010). The superoxide radical is one of the precursors of singlet oxygen and the hydroxyl radical. Superoxide radicals can be generated by pyrogallol auto oxidation (Marklund and Marklund, 1974).

From Fig. 3b, at the concentration from 0.125 mg/mL to 2 mg/mL, the superoxide anion-scavenging activity increase with concentration. It was suggested that inhibition capacity of AAPs towards auto oxidation of pyrogallol was increased, which also meant that auto oxidation rate of pyrogallol was decreased by AAPs. When the concentration was 2 mg/mL, superoxide anion-scavenging activity of AAP2 was 46.96±2.11%, while 98.13±2.30% for asorbic acid.

Hydroxyl radical-scavenging activity: The hydroxyl radical has a very short *in vivo* half-life of approximately 10-9 s and a high reactivity. This makes it a very dangerous compound to the organism. In this experiment, hydroxyl radicals are produced from the decomposition of hydro peroxides. As presented in Fig. 3c, the scavenging capacity of AAP3 was week at low concentration, with only 2.42±0.27% at the concentration of 0.125 mg/mL, but increased quickly with higher concentration. Results indicated that AAP2 had a high level of hydroxyl radical scavenging effect, which is even same as the positive control at 2 mg/mL.

Metal chelating ability: Transition metal iron and copper are the catalysts for many free radicals, especially Fe^{2+}. Fe^{2+} is also a medium for lipid peroxidation and the production of free radicals, such as ·OH. At the concentration from 0.125 mg/mL to 2 mg/mL, metal chelating capacities of AAPs were increased with concentration (Fig. 3d). At the concentration 0.125 mg/mL, the metal chelating capacity of AAP2 was weaker than that of AAP1 (3.14±0.49%; 5.89±0.45%). But when the concentration up to 0.25 mg/mL, the metal chelating capacity of AAP2 was increased rapidly, it was stronger than that of AAP1 (10.95±0.61%; 6.56±0.56%).

Reducing power assay: Reducing power of a compound is also a supporting feature for its antioxidant activity. The reducing properties are generally associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (Geckil *et al*., 2005). In this experiment, the reducing property of AAP was expressed as an A_{700} value, with higher A_{700} meaning stronger reducing activity. In Fig. 3e, A_{700} increased with concentration. Therefore, the reducing power of AAPs was concentration-dependent. But compared with ascorbic acid, AAPs exhibited a weaker reducing power. This suggested that AAPs may contain reductone.

Antioxidant activities of AAP *in vivo*:

Lifespan assay: The *C. elegans* model possesses many advantages over other model systems such as short life cycle, transparent body, low cost and easy handling. The survival curve for N2 worms under normal conditions was in good accordance with those reported previously (Keaney *et al*., 2004; Uchiyama *et al*., 2005). In Fig. 4, treatment with 1 mg/mL AAP3 extended the average lifespan of 4 days ($p<0.05$). On the other hand, AAP3 had slowed the aging of *C. elegans*. Meanwhile, AAP1 and AAP2 did not increase the lifespan. AAP1 did not prolong, but rather reduced the mean lifespan.

Oxidative stress resistance: Oxidative stress was generated in *C. elegans* by exposure to paraquat or H_2O_2, which is an intracellular free radical-generating compound (Castello *et al*., 2007; Wilson *et al*., 2006). In Fig. 5a, treatment of *C. elegans* with 1mg/mL AAP2 and 1mg/mL AAP3 extended the average lifespan by14.3% ($p<0.05$), same as ascorbic acid, whereas AAP1 did not show these effects. When the nematodes were exposed to 0.1 M H_2O_2 for 5 h

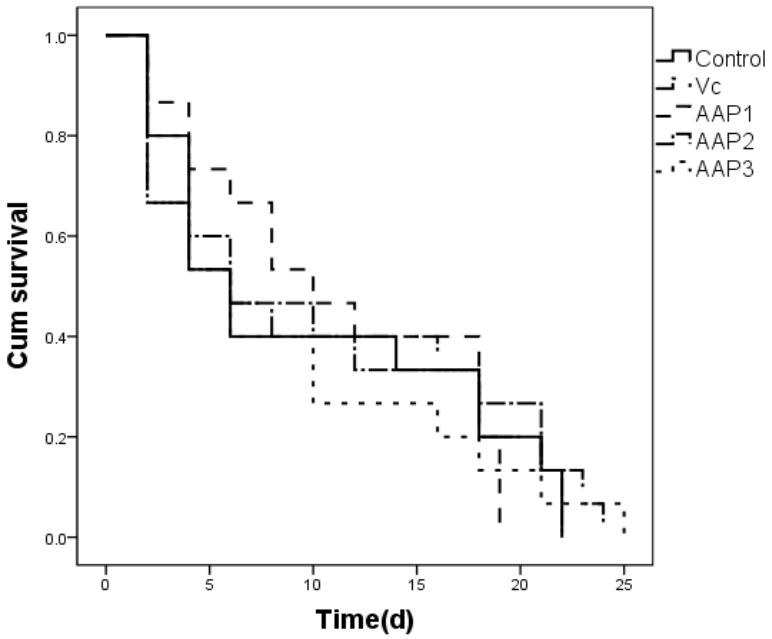
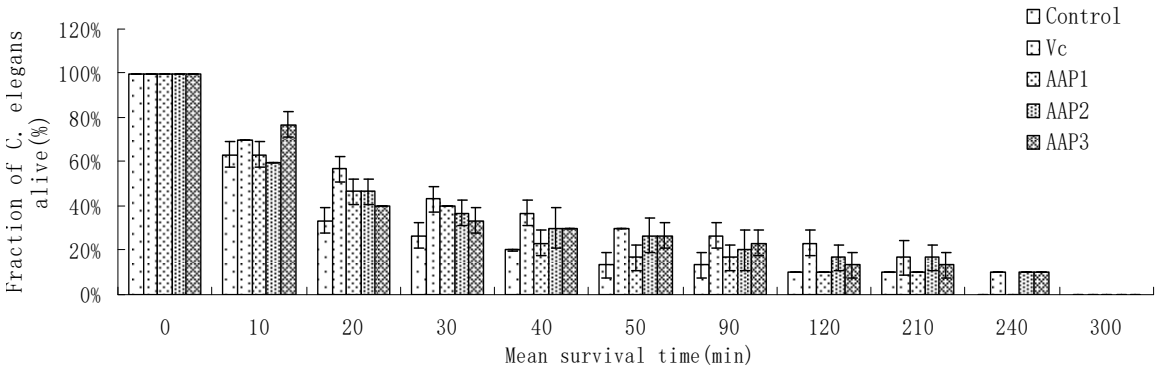


Fig. 4: Effects of AAPs on lifespan of wild-type N2 nematodes. The number of worms used per each lifespan assay experiment was 45–60 (n = 45-60) and three independent experiments were repeated (N = 3)



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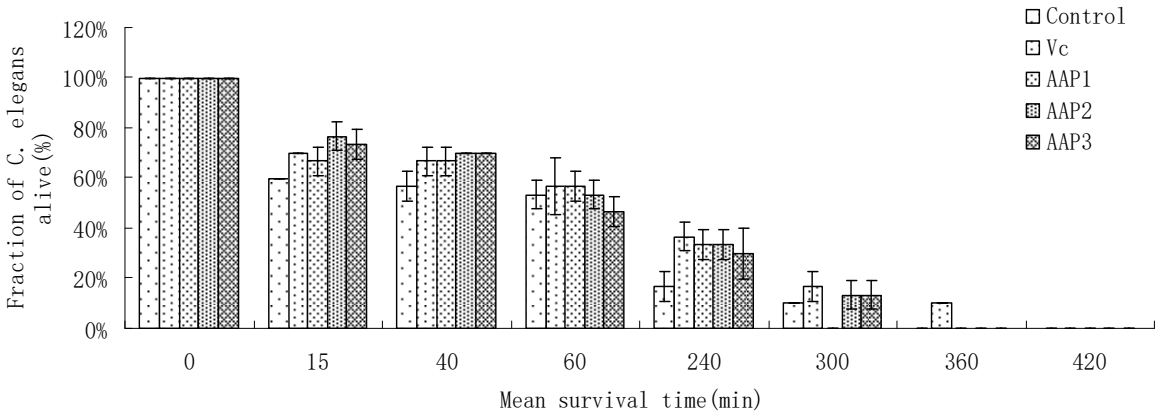


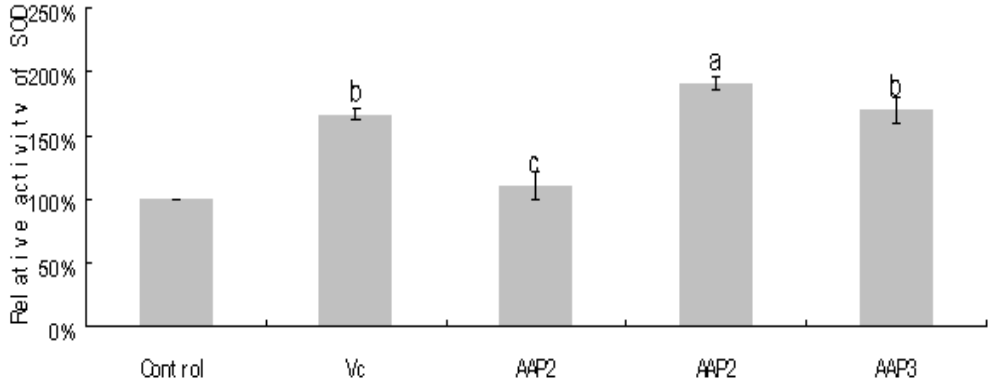
(b)

Fig. 5: Effects of AAPs on resistance to oxidative stress, (a, paraquat-induced; b, H2O2-induced). The number of worms used per each experiment was 15-20 (n = 15-20) and three independent experiments were repeated (N = 3)

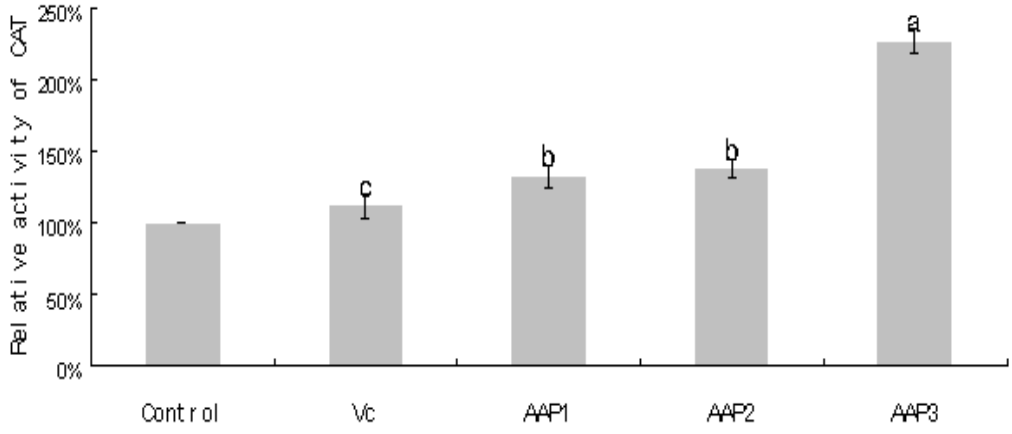
after 2-day treatment with the polysaccharides, the survival of 0.1 mg/mL AAP2 and 0.1 mg/mL AAP3 had been improved but the survival time were not prolonged (Fig. 5b). Meanwhile ascorbic acid significantly increased the mean survival time by 20.0% ($p<0.05$).

Measurement of SOD/catalase activity: The effects of Vc, AAP1, AAP2 and AAP3 on SOD and catalase activities were measured. In Fig. 6a and 6b, three kinds of AAPs had increased the SOD and catalase activities to some extent. To compare the SOD activity of AAPs, their scavenging abilities for $O2^-$ could be seen. In these respects, AAP3 was found to be more potent than the others. Compared with control, the SOD activity of AAP1, AAP2, AAP3 were, 10.55±10%, 91.29±5% and 70.04±10%, respectively (Fig. 6a) and the catalase activity were, 32.49±8%, 37.67±7% and 127.19±8%, respectively (Fig. 6b). These results are consistent with the observations of extension of lifespan, survival time against oxidative stress and lipofuscin accumulation.

Detection of ROS: The ROS levels in *C. elegans* can be examined by molecular probe H2DCF-DA. The fluorescence intensity is proportional to intracellular ROS levels. We measured the effects of AAPs on accumulation of ROS induced by paraquat. Therefore, in Fig. 7, *A. auricula* polysaccharides respectively obtained by three extraction processes reduced the generation of ROS in oxidative stress state of *C. elegans*, while ascorbic acid had a higher ROS level. It was demonstrated that AAP could reduce accumulation of ROS and protect *C. elegans* from the damage of ROS. Treatment with 1mg/mL AAP reduced the level of ROS generated by 83.33 mg/mL paraquat-induced oxidative stress compared with the control (26.0±2.3%, 27.0±2.1% or 9.0±1.6% reduction, respectively, $p< 0.05$). Among them, the AAP2 was more effective than the other two kinds of AAPs at reducing ROS levels, which means that it had stronger ability to protect *C. elegans*.



(a)



(b)

Fig. 6: Effects of AAPs on activity of enzyme (a, SOD; b, CAT) in worms; the number of worms used per each experiment was 70-100 (n = 70-100) and three independent experiments were repeated (N = 3). Figure 6a and 6b mean separation within columns by Duncan's multiple range tests at $p<0.05$. Bars marked without the same letters (a-c) differ significantly ($p<0.05$).

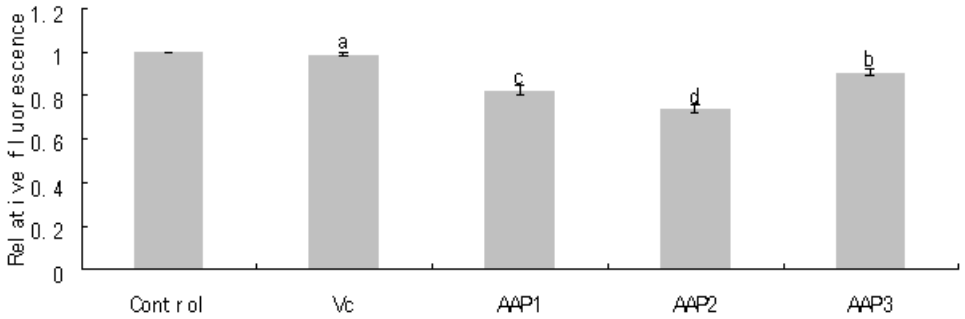


Fig. 7: Effects of AAPs on ROS accumulation in worms; the number of worms used per each experiment was 15-20 (n = 15-20) and three independent experiments were repeated (N = 3). Mean separation within columns by Duncan's multiple range tests at $p<0.05$. Bars marked without the same letters (a-d) differ significantly ($p<0.05$).

CONCLUSION

In the present study, crude polysaccharides from the *A. auricula* were respectively obtained by hot water extraction, enzyme extraction, alkali extraction and then deproteined by Sevag method. The monosaccharide compositions of AAPs were determined by GC method. The evaluation of antioxidant activities *in vitro* and *in vivo* of AAPs was performed. AAPs were observed to have scavenging activity against DPPH, superoxide and hydroxyl radicals. In addition, AAPs exhibited metal chelating ability and reducing power. What's more, their protective effects on paraquat or H_2O_2-induced damage *in vivo* using *C. elegans* were also showed. It was demonstrated that the antioxidant activities *in vitro* and *in vivo* of AAPs were different from extraction processes. Among the three kinds of polysaccharides, AAP2 exhibited the strongest radical scavenging activities and composed of D-arabinose, D-xylose, 2-deoxy-D-arabino-hexose, D-mannose, glucose, N-acetyl-D-glucosamine with the molar ratio of 1:0.44:0.33:1.67:1:0.17.

In conclusion, extraction process is an important factor that affects the antioxidant activity of *A.* auricula polysaccharide. The enzyme extraction is better than the other extraction processes in preparing AAP which has the potential to be a natural polymer antioxidant. However, the antioxidant mechanism of the polysaccharide is still not fully understood. Therefore, further work should be performed to determine the possible antioxidant mechanism.

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