Carbohydrate Polymers 84 (2011) 638–648

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Purified *Auricularia auricular-judae* polysaccharide (AAP I-a) prevents oxidative stress in an ageing mouse model

Hua Zhang [a], Zhen-Yu Wang [a,b,*], Zhi Zhang [b], Xue Wang [c]

[a] School of Food Science and Engineering, Harbin Institute of Technology, 202 HaiHe Road, NanGang District, Harbin 150090, PR China
[b] School of Forestry, Northeast Forestry University, 26 HeXing Road, DongLi District, Harbin 150040, PR China
[c] Symrise (Shanghai) Co., Ltd., Shanghai 201206, PR China

ARTICLE INFO

Article history:
Received 18 May 2010
Received in revised form
12 November 2010
Accepted 15 December 2010
Available online 22 December 2010

Keywords:
Auricularia auricula-judae
Polysaccharide
Oxidative stress
Ageing mouse model

ABSTRACT

This paper reports on a water-soluble polysaccharide (AAP I-a) extracted from *Auricularia auricular* with assistance from ultrasonics, and purified by anion-exchange and gel-permeation chromatography. Additional further structural characteristics were determined from high-performance liquid chromatography/gel permeation chromatograph (HPLC/GPC), Fourier transform infrared (FT-IR) spectrometer and gas chromatography–mass spectrophotometer (GC–MS). AAP I-a is composed of L-rhamnose, L-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose in a molar ratio of 0.2:2.6:0.4:3.6:1.0:0.4. After 35 days of AAP I-a oral administration (50, 100 and 200 mg/kg once a day), the AAP I-a significantly decreased the level of malondialdehyde (MDA) and increased superoxide dismutase (SOD) and glutathione (GSH) activities in mice where ageing is induced by D-galactose ($p < 0.05$). In conclusion, our results indicated that AAP I-a possessed potent antioxidant activity.

1. Introduction

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 & Melov, 2001) because is that oxidative damage should increase with age (Martin & Grotewiel, 2006). Bonnefont, Bastard, Jandon, and Delattre (2000) assert that exposure of organisms to exogenous and endogenous factors generate a wide range of reactive oxygen species (ROS), resulting in homeostatic imbalance. Oxidation is essential to many organisms for the production of energy to fuel biological processes. However, increasing oxidative stress (the uncontrolled production of oxygen-derived free radicals) and disorders in energy metabolisms may lead to mutations and may eventually bring on the onset of many severe diseases. People from all lifestyles, cultures, and backgrounds have always had an interest in some natural products, which have anti-ageing action. Many of these products may work by protecting the human body from various types of oxidative damage that are linked to diabetes, cancer, cardiovascular disease, rheumatoid arthritis, atherosclerosis as well as neurodegenerative diseases such as Parkinsons and Alzheimers (Lin & Beal, 2003; Mau, Lin, & Song, 2002). Almost all organisms have natural antioxidant properties and can repair oxidative damage in their systems, but these systems are unable to avert damage entirely. Antioxidants are substances that can delay or prevent oxidation generally by scavenging free radicals (Sun, Zhang, Zhang, & Niu, 2010). There are synthetic compounds that are strong radical scavengers but usually they have side effects (Zhou & Zheng, 1991). Therefore, research on natural antioxidants, with low cytotoxicity from plants, have become an important branch of biomedicine (Kardošová & Machová, 2006). Edible mushrooms are known to be a highly nutritious food but have also been credited with having tonic and medicinal attributes especially in Chinese folk or traditional medicine. Recently, Chinese researchers and some Western researchers have become interested in finding new functional compounds in mushrooms (Sheu, Chien, Chien, Chen, & Chin, 2004; Yoon et al., 2003).

Some bioactive polysaccharides isolated from natural sources have attracted much attention from researchers in the field of biochemistry and pharmacology (Yang & Zhang, 2009). Particularly, polysaccharides have been demonstrated to play an important role as a dietary free radical scavenger in the prevention of oxidative damage in living organism (Pang, Chen, & Zhou, 2000; Tsiapali et al., 2001). In addition, published data indicates that polysaccharides, generally have a strong antioxidant action and can be employed as novel potential antioxidants (Hu, Xu, & Hu, 2003; Jiang, Jiang,

* Corresponding author at: School of Food Science and Engineering, Harbin Institute of Technology, 202 HaiHe Road, NanGang District, Harbin 150090, PR China. Tel.: +86 451 86282909; fax: +86 451 86282909.
E-mail addresses: wzy219001@yahoo.com.cn, neauxiaoxue@163.com (Z.-Y. Wang).

0144-8617/$ – see front matter.
doi:10.1016/j.carbpol.2010.12.044

Wang, & Hu, 2005; Li, Ma, & Liu, 2007; Ramarahnam, Osawa, Ochi, & Kawaishi, 1995).

Recent literature in this area includes work by Sone, Kakuta, and Misaki (1978), Song, Bao, Li, and Li (1999), Lv, Gu, Tang, and Ho (2007), Lu et al. (2007), Li et al. (2007), Qiao et al. (2009), Ke et al. (2009), and Sun et al. (2007). Together, their research mainly focused on different polysaccharides or other natural products antioxidant activities in mouse model where ageing is induced by D-galactose, in addition to other activities.

This review of the literature also showed that *Auricularia* mushroom is the fourth most important cultivated mushroom in the world (Yan, Luo, & Zhou, 2004). The mushroom *Auricularia auricular-judae* is a traditional Chinese non-toxic edible mushroom widely used in Chinese cuisines; it is also known for its pharmaceutical effects in traditional Chinese medicine. *A. auricula-judae* belongs to the heterobasidiae of the basidiomycete family and is known as a highly nutritious and officinal edible fungus with a high content of carbohydrates and protein (approximately 630 g/kg within the main body of the fruit when dried) (Fan, Zhang, Yu, & Ma, 2006).

In early work Sone et al. (1978); isolated and characterized the polysaccharide of the kikurgae from the fruit body of the *A. auricula-judae*. As well as exploring the structural features, they found and isolated, two kinds of β-D-glucans and an acidic heteropolysaccharide from the fruit body of *A. auricula-judae*, and explored their structural features. In addition, Ukai et al. (1983) researched the polysaccharides found in fungi and the anti-tumor activity of various polysaccharides when isolated from Dictyophora Indusiata, Ganoderma japoncicum, Cordyceps cicadae; their research also included *A. auricula-judae* and *Auricular* sp. They found that an anti-tumor assay provided useful information on the relationship between activity and the structure of the polysaccharides.

More recently, contemporary researchers have researched the biological activity and the benefits to humans of consuming *A. auricula-judae*. Research has found that the fruit of *A. auricula-judae* is rich in hetero-polysaccharides that consist chiefly of D-glucose residue with various chains of β-1, 3-branch residues. The heteropolysaccharide contained mannose, glucose, xylose and glucuronic acid units. It had anti-oxidant activity in vitro, anti-coagulant activity and, as well, the potential to decrease blood sugar levels and aid in the treatment of hypolipidemic, anti-fatigue, and the reduction of atherosclerosis. Again, using experimental mice, researchers have found, when using *A. auricula-judae*, anti-tumor immunomodulation, anti-mutagenic and the protection of the tumor immunomodulation, anti-mutagenic and the protection of the mitochondria of the liver and brain (Fan et al., 2006; Wu, Ding, & Zhang, 2006; Yoon et al., 2003; Zhang & Yang, 1995). Research reported by Chang et al. (1998) has found a methanol extract of *A. auricula-judae* inhibited lipid peroxidation and decreased liver damage in benzo[α] pyrene-treated mice. Mau, Chao, and Wu (2001) found that the methanol extract of *Auricularia polytricha* (*A. polytricha*) had antioxidant activity and prevented lipid oxidation as well as it scavenged radicals and chelated metal ions in vitro. In addition, Koyama et al. (2002) have found that subcutaneous injection of four compounds isolated from *A. polytricha* can reduce acetic acid-induced writhing in mice. Furthermore, Luo et al. (2009) researched anti-oxidative and hypolipidemic properties of a novel functional diet formulation of *A. auricula* and suggested that *A. auricula* could provide future practical application in terms of a functional diet as an adjuvant dietetic food. In relation to the left ventricle ejection fraction in aged mice and from research findings argued by Ma, Wang, Zhang, Zhang, and Ding (2010). Ma et al. (2010) found that water-soluble β-D-glucan from *A. auricular-judae* could be considered as a potential anti-tumor agent and be a candidate for possible future use as an anti-tumor drug. In relation to this finding, Wu et al. (2010) researched the chemical characterization of *A. auricula* polysaccharides and their pharmacological effect on the heart's anti-oxidant enzyme action.

Although it can be concluded that there are many published studies on the health benefits of *A. auricula-judae* in both humans and animals. The review found little research that investigates the *A. auricula-judae's* systematically over the whole cycle from extraction, fraction, purification to its antioxidant activity in vivo, and on changes in the activity of antioxidant enzymes and the immune function. In this paper, by t using cetyl trimethyl ammonium bromide (CTAB) the *A. auricula-judae* water-soluble polysaccharide was separated into two fractions—neutral and acidic. Then following a further fractionation of the acidic polysaccharide by anion-exchange chromatography and gel filtration chromatography it was characterized by HPLC/GPC, FT-IR and GC–MS analysis, followed. Finally, age dependence on changes in the activity of antioxidant enzymes and the immune function to assess (Enhanced Immune Organ Index) the regulatory effects of AAP I-a, when isolated from fruit of *A. auricula-judae* on oxidative stress in an ageing mice model, was investigated.

2. Experimental

2.1. Materials

Grown in Heilongjiang Province, China, the treatment agent, the fruit body of *A. auricula-judae*, was purchased from a Carrefour supermarket (Harbin, China). The *A. auricula-judae* was washed and then oven dried at 70 °C; was then ground to a particle diameter size: 400–500 μm and defatted, by extraction with petroleum ether. The removal of some colored materials treatment with 95% EtOH oligosaccharides and phenolic compounds, followed.

The DEAE-Sephadex A-25 and Sephadex-G200 were both purchased from Amersham Pharmacia Company (Sweden). Trifluoroacetic acid (TFA) was purchased from E. Merck, Darmstadt, Germany. D-Mannose, L-rhamnose, D-glucuronic acid, D-glucose, D-xylose, D-galactose, and L-arabinose were all purchased from Sigma, St. Louis, USA, and the T-series Dextrans from Agilent, Beijing, China. The superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-px) commercial kits for detecting enzyme activation were all purchased from Jiancheng Institute of Biotechnology, Nanjing, China. Other reagents were of analytic reagent grade.

2.2. Animals

Fifty male Kunming mice weighing 18–22 g were purchased from Tumor Hospital Experimental Animal Center of Harbin Medical University, Heilongjiang Province (China). Before commencement of the research, all mice were kept in stainless cages under a constant 12 h light/dark cycle at 25 °C and were then acclimatized for a period of a week while at the same time given free access to food and water.

2.3. Extraction of polysaccharide (AAP) from A. auricular-judae

The resulting *A. auricula-judae* was weighed and then immersed in 100 volumes of clean distilled water at room temperature for 24 h. It was then homogenized by a high-cutting dispersible mulser for 1 min. Then followed an ultrasonic-assisted extraction by hot water at 100 °C for 5 h. The residue was extracted twice using the same procedure. The resulting suspension was then centrifuged (4000 r/min for 10 min). It was then concentrated in a rotary evaporator under reduced pressure at 50 °C. The concentrated supernatants were then precipitated with three volumes of absolute ethanol (95%) and maintained at 4 °C overnight. The resulting precipitate was then separated by centrifugation, washed

exhaustively with 95% alcohol, dissolved in deionized water, and dialyzed using cellulose sacks (Sigma). The non-dialyzed portion was lyophilized, to give the crude polysaccharide extract. This preparation, for the purpose of this research, is called 'crude polysaccharide' (Zhang, Cui, Cheung, and Wang, 2007).

As a final point in the preparation, hydrogen peroxide (H_2O_2) was added, one drop at a time, to the prepared 0.5% crude polysaccharide solution of *A. auricula-judae*. The final concentration of hydrogen peroxide (H_2O_2) reached a 3%, water bath solution and was then set at 45 °C for 3 h; after which time this preparation was subjected to a reverse tap water flow dialysis for 48 h. Then followed, for another 24 h, a distilled water dialysis vacuum concentration, which embraced a 95% ethanol mix prepared at three times the volume of the concentrated supernatants. Then followed a precipitation process with ethanol, acetone, and ethyl ether washing, followed by freeze-drying at the end of this procedure the polysaccharide of *auricula-judae* was obtained which was further purified by the CTAB fractionation process.

2.4. Fractionation of AAP using cetyl trimethyl ammonium bromide (CTAB)

Yang and Zhang (2009) reported that the separation of an acidic and a neutral polysaccharide is possible using (CTAB) or cetylpyridinium chloride (CPC), which form an insoluble precipitated complex with the acidic polysaccharide. This finding justified the use of CTAB as outlined below.

2.5. Further fractionation of acidic polysaccharide from A. auricula-judae

Further fractionation was performed using anion-exchange chromatography. The acidic polysaccharide of *A. auricula-judae* obtained by CTAB was dissolved in a Tris–HCl buffer and then membrane-filtered (0.45 μm). The solution was then applied to a column (1.6 cm × 60 cm) of DEAE-Sephadex A-25 (Amersham Bio Sciences) pre-equilibrated with a Tris–HCl (0.02 mol/L, pH 7.4) buffer. Fractions were prepared in stepwise elution with increased ionic strength of NaCl (0, 0.1, 0.3, and 0.5 mol/L) at a flow rate of 0.4 mL/min. The main fraction from the eluted Tris–HCl solution, which quantified by the phenol–sulfuric acid method as described previously (Zhang, 1999). It was then collected and further purified by gel filtration chromatography on a Sephadex-G200 column (1.6 cm × 60 cm). The sample was then dissolved in the minimal volume of 0.1 mol/L NaCl solution and added to the column; and eluted with 0.1 mol/L NaCl (0.02 mol/L Tris–HCl, pH 7.4) at a flow-rate of 0.2 mL/min. The major fractions obtained were pooled, concentrated, and dialyzed against deionized water with dialysis tubing (molecular weight cut-off, 3500 Da), concentrated, and then lyophilized to give the *A. auricula-judae* polysaccharide coded as AAP I-a.

2.6. Determination of molecular weight—molecular weight distribution

The molecular weight (M_w) of AAP I-a was measured by high-performance gel-permeation chromatography (HPLC/GPC) on an Agilent 1100 HPLC system (Agilent, USA) equipped with a differential refractive index detector (RID). As well, an automatic sample injector was used. All separations were performed with a PL Aquagel-OH mixed column (300 mm × 7.5 mm i.d. × 8 mm) and then the retention time were plotted against the logarithms of their corresponding average molecular weights. All spectra were acquired by ChemStation software system (Agilent, USA). The mobile was 0.02% NaN_3 water solution and with a flow-rate of 1.0 mL/min. Column temperature is 30 °C. A 50 μL sample passed through a 0.45 μm cellulose acetate filter was injected in each run. The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (106, 194, 620, 1470, 4120, 11,840, 25,820, 58,400, 124,700, 465,000, 965,000, and 1,250,450 Da).

2.7. Infrared spectroscopy analysis

An infrared (IR) analysis of the *A. auricula-judae* polysaccharide was obtained by grinding a mixture of polysaccharide with dry KBr and then pressing the mix into a mould. The IR spectra recorded on the spectrum one FT-IR spectrometer (PerkinElmer, USA) was run in the 4000–400 cm^{-1} region.

2.8. Determination of constituent monosaccharides using a gas chromatography–mass spectrophotometer (GC/MS)

The monosaccharide samples were prepared according to the method defined by He et al. (2007) and Liu, Wang, Xu, and Wang (2007). With slight modifications 10 mg of dried AAP I-a, was hydrolyzed with 2 mol/L of trifluoroacetic acid (TFA) at 120 °C for 3 h. It was then centrifuged at 5000 r/min at room temperature for 10 min. The supernatant followed by evaporation in a stream of nitrogen was then dissolved in 0.5 mL of pyridine, after which 10 mg hydroxylamine hydrochloride and 7 mg inositol hexaacetate was added. This mixture was allowed to react at 90 °C for 30 min, it was then allowed to cool to room temperature. For acetylation, 0.5 mL of acetic anhydride was added at 90 °C, the reaction process was then allowed to continue for another 30 min.

The prepared acetylated aldononitriles, were then passed through a nylon filter and analyzed using an Agilent 6890N Gas Chromatograph/5973 Mass-Selective detector with a capillary column-DB-5, 60 m × 0.25 mm i.d. × 0.25 μm.

Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The temperature program was set to increase from an initial 120 to 200 °C at 8 °C/min and held for 10 min and then heated at 6 °C/min to 230 °C and then held for a further 20-min. Peaks were identified and estimated using myoinositol as the internal standard. The quantity of fractions was determined from the peak area, using response factors. The inlet temperature was kept constant at 210 °C, and the MS transfer line was set at 270 °C. Mass spectrophotometer (MS) acquisition parameters included scanning from m/z 50 to 550 in the electron impact (EI) mode for routine analysis.

2.9. Treatment of antioxidant activity of AAP I-a in vivo

2.9.1. Animal treatment

With some modifications, the assay of anti-oxidant activity in vivo of AAP I-a was carried out in accordance with the method adopted by Li et al. (2007), Sun et al. (2007), Su, Wang, and Liu (2009) and Ke, Lin, Chen, Ji, and Shu (2010). Male Kunming mice (8 weeks old) weighing 18–22 g were purchased from Harbin Medical University Cancer Hospital Experimental Animal Center, China. All 50 mice were fed a standard laboratory diet, given distilled tap water, and maintained under a constant 12 h of light and dark cycle at 25 °C. Before treatment, all mice were acclimatised for a period of a week in stainless steel cages. At the same time, they were randomly divided into five groups ($n = 10$) and housed in individual pens with sawdust bedding. Group I was the control group, the other 40 experimental group mice were the D-galactose treated mice. The experimental group mice, known as Groups II, III, IV, and V (sub-acute D-galactose induced mouse-ageing model) were given a subcutaneous injection to the back at a dose rate of 100 mg/kg body weight (BW) of D-galactose once daily for 35 consecutive days. The mice were weighed every 2 days. According to their weight, and to meet 100 mg/kg BW/day for D-galactose and the 50, 100 and

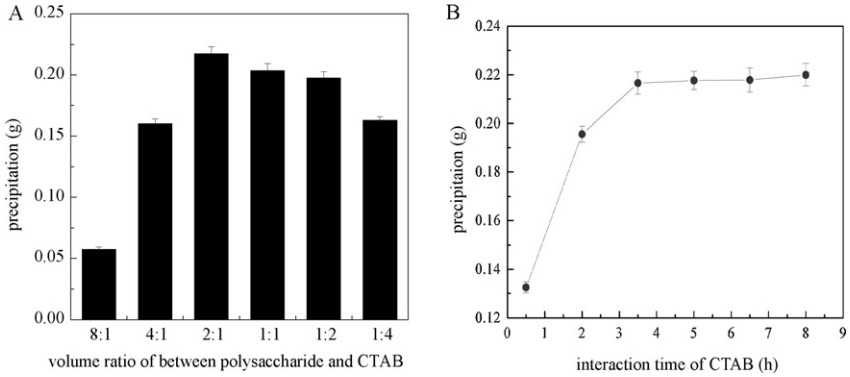

Fig. 1. The effect of different factors on acidic neutral polysaccharides production. *Auricularia auricular-judae* crude polysaccharides were separated into acidic and neutral polysaccharides by using (CTAB). (A) Volume ratio of between polysaccharide and CTAB effects on acidic polysaccharides production. (B) Interaction time of CTAB effects on acidic polysaccharides production.

200 mg/kg target for AAP I-a. Simultaneously, the ageing control group (Group II) mice, were each orally administered with 0.3 mL of physiological saline, respectively, and were fed with a standard mice food for 35 days. The normal control group (Group I) mice were orally administered with 0.3 mL of physiological saline. In addition, the polysaccharide treated mice Groups III, IV, and V, were orally administered with a different dose of AAP I-a at 50, 100 and 200 mg/kg, respectively.

2.9.2. Assay of SOD, GSH-Px, and MDA

Following the 35-day treatment process and 24 h after the last administration, all 50 mice were weighed then sacrificed by the humane method of cervical dislocation after which the eyes of these mice were surgically removed. Their blood was then collected and centrifuged at 10,000 rpm/min at 4 °C for 10 min to obtain blood serum which was then stored at −80 °C for further analysis. Following this procedure, the organs (heart and liver) were surgically excised from the animal, accurately weighed, and then homogenized immediately in ice-cold 0.9% NaCl solution (0.1 g tissue/mL solution). The suspension was centrifuged at 4000 rpm/min at 4 °C for 10 min, and the supernatant was collected for further analysis. The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and the level of malondialdehyde (MDA) were measured using commercially available kits (Nanjing, Jiancheng, China) and in accordance with the commercial kit manufacturer's instructions (Ma, Liu, Yu, Chen, & Zhang, 2009). All the above treatments were performed at 4 °C.

One unit (U) of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%, and the data were presented as U/mg protein. The activity of GSH-Px was determined by quantifying the catalyzed reaction rate of GSH per minute on the base of its catalysis. The outcome was expressed as a decrease of 1.0 μmol/L GSH per 5 min at 37 °C after the non-enzymatic reaction was subtracted and the data were then expressed as U/mg of protein. All the above enzymatic activities were expressed as unit per milligram of protein (U/mg protein) in the liver and heart or a unit per millilitre in the blood serum (U/mL). Similarly, the MDA level was measured by using the 2-thiobarbituric acid (TBA) method and expressed as a unit per milligram of protein (U/mg protein) in the liver and heart or a unit per millilitre in the blood serum (U/mL).

2.9.3. Analysis of thymus and spleen indices

To analyze the index of the spleen and thymus, the spleen and thymus of the mice were also surgically removed and weighed. The thymus and spleen indices were calculated and in accordance with the following formula according to the method as described previously (Ma et al., 2009; Zalys, Zagon, Bonneau, Lang, & McLaughlin, 2000):

$$\text{Thymus or spleen index} = \frac{\text{weight of thymus or spleen}}{\text{body weight}} \times 100$$

2.10. Statistical analyses

All data in the illustrated figures and tables that follow are presented as mean ± S.D. ($n = 10$) and differences between groups were assessed by an analysis of variance (ANOVA) using both directional and non-directional hypothesis t-tests. Differences were considered to be statistically significant, if $p < 0.05$. All statistical analyses employed SPSS for Windows, Version 13.0.

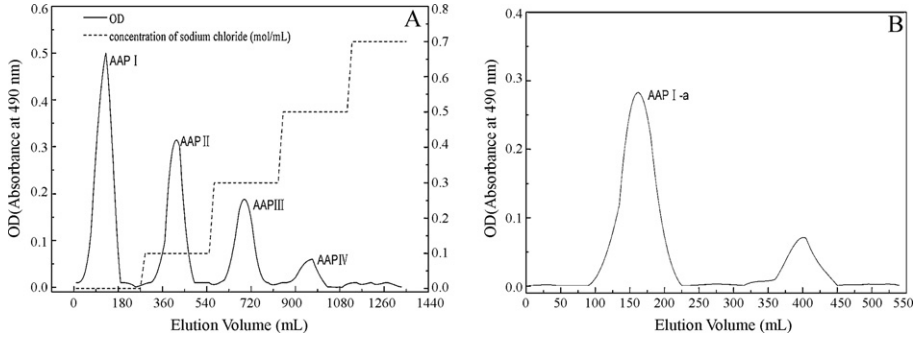

Fig. 2. Fractionation of acidic AAP. The acidic *A. auricula-judae* polysaccharide was further separated by DEAE-Sephadex A-25 column chromatography into four fractions, namely AAP I, AAP II, AAP III, and AAP IV (A). The main peak (AAP I) was further fractionated over Sephadex-G200 column and collected main component, namely AAP I-a (B).

3. Results

3.1. Purification of AAP I-a

3.1.1. Fractionation of AAP using CTAB

This section describes the results from the fractionation of AAP using CTAB together with the preparation for analysis of the purity of the polysaccharide fractionation. As shown in Fig. 1A, when the AAP polysaccharide was twice the volume of CTAB, the precipitation reached a maximum of 0.2174 g, as illustrated in Fig. 1B, the polysaccharide was twice the volume of CTAB and that the reaction time of 3.5 h was the optimal parameter to obtain maximum acidic AAP using CTAB.

3.1.2. Further purification of the acidic polysaccharide fractionation

The acidic *A. auricula-judae* polysaccharide was further separated by DEAE-Sephadex A-25 column chromatography into four fractions by stepwise elution with Tris–HCl (0.02 mol/L, pH 7.4) and sodium chloride solutions (0, 0.1, 0.3, and 0.5 mol/L), namely AAP I, AAP II, AAP III, and AAP IV (Fig. 2A). The material recovered from the main peak (AAP I) was further fractionated over Sephadex-G200 column (Fig. 2B), which stressed that the AAP I-a was a major polysaccharide of the AAP I in the acidic *A. auricula-judae*.

3.2. Molecular mass distribution of AAP I-a

A typical size-exclusion HPLC chromatogram of AAP I-a, gave only a single and symmetrically sharp peak region: 5.00–10.00 min (Fig. 3A). The retention time of AAP I-a was then plotted on the same graph and weight-average molecular weight and number-average molecular weight of the AAP I-a, when referenced to a standard dextrans, were 2.06×10^5 and 7.41×10^4.

3.3. Fourier transform infrared (FT-IR) spectroscopy analysis (FT-IR)

FT-IR spectra of these polysaccharide showed the characterization of polysaccharide. FT-IR spectra (Fig. 3B) obtained from *A. auricula* polysaccharide had peaks at about 3500 cm^{-1} and 500 cm^{-1} in the carbohydrate region. These are the characteristic of polysaccharide. In all spectra, the absorption between 1735 cm^{-1} and 620 cm^{-1} may be attributed to bands of C=O, C–H, C–O and O–H in the polysaccharide. This result revealed that there is a pyranose ring in the AAP I-a polysaccharide and that this was a type of acidic polysaccharide.

3.4. Gas chromatography–mass spectrometry analysis (GC–MS)

The monosaccharide component of AAP I-a, together with a comparison with a number of other researcher's results are shown in Table 2.

3.5. The effect of AAPI-a administration on mice body weight, spleen and thymus indexes

In the present study, the effect of AAPI-a administered on the body weight of mice was conducted. Table 3 summarises the effects of polysaccharide administration on body weight of mice relative to the control mice (Group I); who were not drug treated. Polysaccharide administration did not enhance body weight of mice (Groups I, II, III, IV and V) and, as well, the mice suffered no ill effects.

As shown in Table 3, it was found that the spleen and thymus indexes in aged model Group (II) were lower than those in the aged control group mice (Group I) ($p < 0.05$). Independent treatment with the polysaccharide significantly increased the spleen and thymus indexes in aged mice (Groups III, IV and V) compared to the younger Group II, mice ($p < 0.05$ and $p < 0.01$). Polysaccharide treatment at doses of 100 and 200 mg/kg/day implies that the AAPI-a effectively stimulated the immune system of aged mice.

3.6. The antioxidant activities of AAP I-a in vivo

3.6.1. The effect of AAP I-a on the activities of SOD, GSH-Px and MDA in the liver of aged mice

The effect of AAP I-a on the activities of SOD, GSH-Px and the levels of MDA in the liver of aged mice is shown in Table 4. A marked increase in MDA and significant decrease ($p < 0.05$) of antioxidant enzymes activity (SOD, GSH-Px) was observed in the liver between the treatments of Group I (Normal Control Group) and Group II (Model Control Group). The AAP I-a treatment significantly inhibited ($p < 0.05$) the formation of MDA in the liver and raised the activity of antioxidant enzymes in a dose-dependent manner (Groups III and IV). The administration of AAPI-a to the D-galactose treated mice (Groups II, III, IV and V) with 100 and 200 mg/kg increased both SOD and GSH-Px enzymatic antioxidant liver activity ($p < 0.01$ and $p < 0.05$).

3.6.2. The effect of AAP I-a on the activities of SOD, GSH-Px and MDA in the aged mice blood serum

As shown in Table 4, the administration of AAP I-a elevated the activities of antioxidant enzymes (SOD and GSH-Px), while at the same time it reduced the level of MDA in the blood serum (Groups III to V). Therefore, it was concluded that AAP I-a may reduce oxidative stress and ageing phenotypes by increasing SOD and GSH-Px activities, as well as reducing the level of MDA, although these mechanisms remain to be clarified. In this test, probability value ($p < 0.05$) and ($p < 0.01$) was also observed in blood serum treatments of AAP I-a; meaning that the total antioxidant capability of the antioxidant systems in organism had been enhanced.

3.6.3. The effect of AAP I-a on the activities of SOD, GSH-Px and MDA in the aged mice heart's

This section reports on the effect of AAP I-a on the activities of antioxidant enzymes in the hearts of aged mice. Table 4 shows these results. To explain the mechanism of purified *A. auricula* polysaccharide (AAP I-a) in improving heart function; examined were the antioxidant enzyme activities of each mouse's heart together with the MDA level.

4. Discussion

As shown in Fig. 1A, when the AAP polysaccharide was treated with eight volumes of CTAB, the precipitation production was 0.0573 g. With increasing volumes of CTAB increasing, the precipitation still increased. First, when the AAP polysaccharide was twice the volume of CTAB the precipitation reached a maximum of 0.2174 g, and then with the CTAB continuously increased, the precipitation decreased to such an extent that when the CTAB was four times the volume of the polysaccharide, the amount of precipitation had decreased to 0.1630 g. As a cationic surfactant, the CTAB was not only utilised to precipitate the acidic polysaccharide in the low ionic strength solution, but also employed to combine with the polysaccharide in addition to the acidic polysaccharide in the high-ionic strength solution.

As illustrated in Fig. 1B, with the reaction time extended, precipitation gradually increased. At first, the precipitation increased quickly from 0.5 to 3.5 h. With the time extending to 3.5 h, the precipitation increased slowly from 0.2166 to 0.2200 g. Using CTAB, the results demonstrate that the polysaccharide was twice the volume of CTAB and that the reaction time of 3.5 h was the optimal parameter to obtain maximum acidic AAP using CTAB.

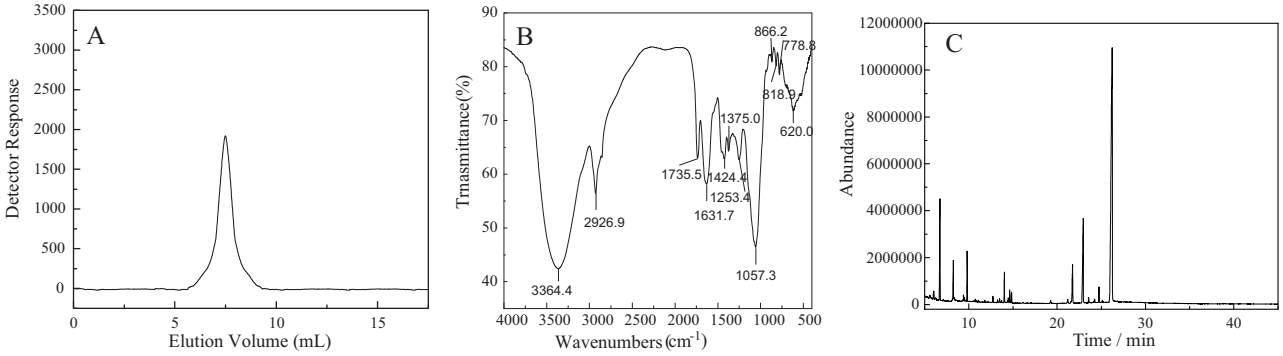

Fig. 3. Chemical analysis of AAP I-a. (A) GPC chromatography of AAP I-a; (B) FTIR spectra of AAP I-a; (C) GC–MS analysis of AAP I-a.

Chromatography on a DEAE-Sephadex A-25 column was carried out to obtain the preliminary chemical information about the acidic polysaccharide extracts evaluated in the later pharmacological assay. The acidic *A. auricula-judae* polysaccharide complex was found to be substantial from the point of view of therapeutic applications. It was further separated by DEAE-Sephadex A-25 column chromatography into four fractions by stepwise elution with Tris–HCl (0.02 mol/L, pH 7.4) and sodium chloride solutions (0, 0.1, 0.3, and 0.5 mol/L). Acidic AAP was resolved into four distinct peaks, namely AAPI, AAP II, AAP III, and AAP IV (Fig. 2A). In addition, absorbance peaks of nucleic acid and protein were not detected between the wavelengths of 200 and 400 nm by the UV detector. Together, this confirms that the purified fraction did not contain nucleic acid and protein impurity and is of high purity. The material recovered from the main peak (AAP I) was further fractionated over the Sephadex-G200 column (Fig. 2B), which emphasized that the AAP I-a was a major polysaccharide of the AAP I in the acidic *A. auricula-judae* polysaccharide. The major fractions obtained were pooled, concentrated, and dialyzed against distilled water with dialysis tubing (molecular weight cut-off, 3500 Da), concentrated, and lyophilized to give a brown polysaccharide material (AAP I-a). This result demonstrates that the purified polysaccharide, that results from the acidic polysaccharide extracts from *A. auricula-judae* is composed of a mainly homogeneous component.

A typical size-exclusion HPLC chromatogram of AAP I-a, gave only a single and symmetrically sharp peak region: 5.00–10.00 min (Fig. 3A). This result indicated that AAP I-a, was a homogeneous polysaccharide and did not contain other material. The retention time of AAP I-a was then plotted on the same graph and the weight-average molecular weight and the number-average molecular weight of the AAP I-a, when referenced to a standard dextrans, were 2.06×10^5 and 7.41×10^4. When compared with the results described in Section 1, the molecular weight of AAP I-a correlated but did not completely not agree with agree with Zhang, Yang, Ding, and Chen (1995), Zhang, Yang, and Chen (1995) and Zhang and Yang (1995). In conclusion, the experimental results suggest that there should have been a certain relationship with several factors such as the origin of *A. auricula-judae* extraction purification.

An FT-IR spectroscopy analysis was used to investigate the vibrations of molecules and polar bonds between the different atoms. According to Yang and Zhang (2009), it is possible to analyze the structures of polysaccharide, such as monosaccharide types, glucosidic bonds, and functional groups using an FT-IR spectroscopy.

The most representative bands in the 3800–2700 cm^{-1} are those assigned to the hydroxyl (O–H) intra-molecular and intermolecular stretching modes and to the asymmetric and symmetric methyl and methylene stretching (Popescu et al., 2009). The AAP I-a in the frequency range of 4000–400 cm^{-1} were then ground and blended with a KBr powder which was then pressed into pellets for the FTIR measurement (Fig. 3B). In all spectra, the band in the region of 3364.4 cm^{-1} corresponds to the hydroxyl stretching vibration of the polysaccharide and at 2926.9 cm^{-1} corresponds to a weak C–H stretching vibration. The band 1735.5 cm^{-1} that in turn corresponds to the stretch vibration of C=O. The band in the region of 1631.7 cm^{-1} corresponds to associated water. In addition, a characteristic absorption at 1400–1395 cm^{-1} was also observed and corresponds to C–H stretching vibration of carboxyl of the sugar units, which signifies that the AAPI-a was an acidic polysaccharide (Table 1).

The wave number between 950 and 1200 cm^{-1} is often called the fingerprint of molecules. It allows the identification of major chemical groups in polysaccharide that are the position and intensity of the bands specific for each polysaccharide (Fellah, Anjukandi, Waterland, & Williams, 2009; Ke et al., 2009; Mao et al., 2009; Popescu et al., 2009; Qiao et al., 2009). Fig. 3B illustrates that the absorption at 1057.3 cm^{-1} and 1253.4 cm^{-1} corresponds to C–O–C and O–H of pyranose of sugar units and verified that the AAP I-a indicated α-pyranose of the glucose (He et al., 2007; Liu, Lin, et al., 2007; Zhang, 1999; Zhao, Kan, Li, & Chen, 2005). The band in the region of 1000–700 cm^{-1} corresponds to a α,β-pyranose monosaccharide. The high intensity of the band around 1730 cm^{-1} illustrates that the glucose content in the polysaccharide was higher (Ke et al.,

Table 1
The stretching vibration (cm^{-1}) of FTIR spectra of AAP I-a.

Absorption at region	Vibration type	Functional group
3364.9	Stretching vibration of O–H	O–H
2926.9	Stretching vibration of C–H	$-CH_2$
1735.5	Stretching vibration of C=O asymmetric stretching vibration of C=O	–COOR associated water
1631.7	Variable angle vibration of N–H	–CONH
1424.4	Stretching vibration of C–O	–COOH
1057.3	Stretching vibration of C–O–C and C–O–H	Pyran ring
778.8	Symmetric stretching vibration of C–O–C	D-Glucose pyranose
818.9		αD-galactose pyranose
866.2		βD-glucose pyranose

Table 2
Monosaccharide component of polysaccharides from *Auricularia auricular-judae* researched by researchers.

Reference	Original	Preparation	Monosaccharide composition							
			L-Fucose	L-Rhamnose	L-Arabinose	D-Xylose	D-Mannose	D-Glucose	D-Glucuronic acid	D-Galactose
The molar ratios were (Sone et al., 1978)	Dried fruit body of *A. auricula-judae*	Isolated from the hot water extract through insoluble complex formation with cetylpyridinium chloride.	–	–	–	1.0	4.1	1.3	1.3	–
The molar ratios were (Xia and Chen, 1988)	The fruit bodies of the *Auricularia*	Isolated from the hot water extract and deproteinized by sevage	0.14	–	0.045	0.17	1.00	0.61	0.44	–
Molar percentage were (Ding, 1994)	Yuexi County, Anhui Province, China	Isolated from the hot water extract and purified by sephadex G-100	18.6%	12.8%	–	5.6%	25.7%	9.4%	–	15.7%
The molar ratios were not characterized (Zhang, Yang, Ding, et al., 1995	Fang city, Hubei province, China	Polysaccharides B was extracted with NaOH. Extraction with hot water, was precipitated with EtOH, precipitate was desoluted with Na_2SO_4 solution precipitated with CPC, named as D	–	–	–	–	–	–	–	–
The molar ratios were (Fan, 2006)	Fang city, Hubei province, China	Extracted by water (ultrasonic wave combined with enzyme method), deproteinized by sevage, purified by DEAE Sephadex A-25 and Sephadex G-200 column, named as AAP-II a	1.27	0.23	–	–	1.00	11.52	0.61	–
The content of glucuronic acid was (Ma et al., 2008)	Hubei province, China	A water soluble β-D-glucan, named as AAG	–	–	–	–	–	–	19%	–
The molar ratios were not characterized (Chen et al., 2008)	DaXing'an Mountain range, Heilong, Jiang province, China	Prepared according to the method reported by Mizuno et al. (1992)	–						–	
The ratio were (Wu et al., 2010)	Changsha city, Hunan province, China	Isolated from the hot water extract and removed small molecular substances using an ultraltration system	10%	–	–	10%	8%	72%	–	–

"–" means not detected.

Table 3
Effects of AAP I-a administration on body weight (B.W.) and the immune organ indexes of mice.

Group	Dose (mg/kg/day)	Initial B.W. (g)	Finial B.W. (g)	Thymus index (%)	Spleen index (%)
I		20.59 ± 0.57	33.75 ± 1.54	0.425 ± 0.008[a]	0.542 ± 0.011[b]
II		20.73 ± 0.49	33.5 ± 1.13	0.382 ± 0.010	0.435 ± 0.017
III	50	20.72 ± 0.73	33.26 ± 1.56	0.395 ± 0.004	0.485 ± 0.018[b]
IV	100	20.69 ± 0.31	33.56 ± 1.31	0.415 ± 0.011[a]	0.474 ± 0.013[b]
V	200	21.06 ± 0.61	33.77 ± 1.81	0.423 ± 0.018[a]	0.527 ± 0.023[b]

Data were expressed as mean ± S.D. ($n = 10$) and compared using a non-directional hypothesis two-tailed t-test; $p < 0.05$ was taken as statistically significant. A Duncan's Multiple Range Test determined which sample means differed significantly from one another. Control and aged model mice (I, II) were fed with a standard mice chow for 35 days. Three groups of polysaccharides-treatment mice (III, IV, V) were each orally given the polysaccharide in a single dose of 50 mg/kg, 100 mg/kg and 150 mg/kg bodyweight once daily were, at the same time, fed with standard mice consisting mainly of grain for 35 days.
B.W.: body weight.
[a] $p < 0.05$ compared with aged model.
[b] $p < 0.01$ compared with aged model.

2009). In view of the fingerprint spectra of AAPI-a, it is postulated from the results that the AAPI-a is closely related to acidic heteropolysaccharide family with certain uronic acid and higher glucose content and D-pyranoses (Ma, Wang, & Zhang, 2008).

The monosaccharide component of AAP I-a, together with a comparison of a number of other researcher's results when doing a similar (GC–MS) analysis are as follows. The acetylated aldononitriles of the AAPI-a monosaccharide were analyzed by the previously described GC–MS method under the optimized conditions using six monosaccharide standards as the standard measure. A characteristic of the chromatogram depicted in Fig. 3C demonstrates that the AAPI-a derivative of the component monosaccharide released from the polysaccharide sample could be baseline separated and that the component monosaccharide may well be identified by a comparison with the chromatogram of the mixture of a standard monosaccharide (Fig. 3C).

Table 2 shows the monosaccharide component of polysaccharide from *A. auricular-judae* researched by researchers including our group. Most polysaccharide from *A. auricular-judae* contained D-xylose, D-mannose, D-glucose, D-glucuronic acid, L-arabinose, D-galactose, L-fucose and L-rhamnose although species and origin of experimental materials (*A. auricular-judae*) differ.

In common with different preparations of each polysaccharide, there may be a difference in the monosaccharide component of polysaccharide from *A. auricular-judae*. Nonetheless, when compared with other researchers' results, our research results showed that the polysaccharide was a typical hetero-polysaccharide and composed of L-rhamnose, L-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose, with the molar ratio at 0.2:2.6:0.4:3.6:1.0:0.4, respectively. Our research clearly indicated that the predominant composition monosaccharide within the polysaccharide were neutral D-mannose, L-arabinose residues with low amounts of L-rhamnose residue and without D-glucuronic acid, and L-fucose residue.

In the present study, the effect of AAP I-a administration on the body weight of mice was conducted. Table 3 summarises the effects of polysaccharide administration on body weight of mice relative to the control mice (Group I) who were not drug treated. During the initial days of feeding, there was no significant difference in body weight between control Group (I) and the experiment Groups (II to V) ($p > 0.05$) (Table 3). This difference in body weight between the control and experimental groups had persisted from the 3rd day throughout the final feeding period (35 days). However, no significant differences were found between the body weights of untreated and polysaccharide-treated mice at the end of the polysaccharide treatment period. Polysaccharide administration did not enhance body weight of mice (Groups I, II, III, IV and V) and the mice suffered no harm or ill effects.

When compared with the control group, values are the mean standard deviation (SD) of 10 parallel measurements. Control and aged model mice (Groups I and II) were fed a standard mice food for 35 days. Three groups of polysaccharide-treated mice (Groups III, IV, and V) were orally given an intragastric gavage of polysaccharide in a single dose of 50, 100, and 150 mg/kg body weight respectively once daily and were fed with standard mice food for 35 days.

A variety of immune changes occurs in both animals and humans with increasing age. The ageing of the immune system (immunosenescence) is associated with dramatic reductions in immune responsiveness as well as functional deregulations (Ke et al., 2009). As shown in Table 3, it was found that the spleen and thymus indexes in the aged model Group (II) were lower than those in the aged control group mice (Group I) ($p < 0.05$). Independent treatment with the polysaccharide, significantly increased the spleen and thymus indexes in aged mice (Groups III, IV and V) compared to the younger Group II, mice ($p < 0.05$ and $p < 0.01$). Polysaccharide treatment (dose-rate: 100 and 200 mg/kg/day) implies that the AAPI-a effectively stimulated the immune system of aged mice. The decrease in thymus and spleen indices in aged mice is a good indicator of age-induced decline in immune function. Although the exact mechanism for the immune-stimulating activity of the AAP I-a is not known, it is proposed that it may act by inducing of the antioxidant enzymes SOD and GSH-Px.

SOD protects against oxygen free radicals by catalyzing the removal of the superoxide radical, which damages the membrane and biological structures. GSH-PX catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH and MDA. Their variety in the level in laboratory aged mice among the control aged model mice (Groups I and II) and three groups of polysaccharide-treated mice (Groups III, IV, and V) is related to the antioxidant activities of AAPI-a.

D-Gal can cause accumulation of reactive oxygen species (ROS), or stimulate free radical production indirectly by the formation of an advanced glycation end-product (AGE) in vivo. AGE cannot be metabolized further and accumulate in neurons to amplify oxidative stress. Further studies show that ageing-related changes induced by D-Gal include the increase of free radicals and the decrease of antioxidant enzymatic activity (Lu et al., 2007; Qiao et al., 2009; Song et al., 1999). The biological redox substance in mice can be disturbed by long-term injection of D-galactose (Su et al., 2009). SOD protects against oxygen free radicals by catalyzing the removal of the superoxide radical, which damages the membrane and biological structures. GSH-PX catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH (Lv et al., 2007). MDA, the main product of lipid peroxidation, is an indicator of lipid peroxidation. A lower MDA level suggests that there is less lipid peroxidation and weaker oxidant stress (Bagchi, Bagchi, Hassoun, & Stohs, 1995).

The results of AAPI-a on the effect of antioxidant activity in the liver of aged mice is now discussed in detail. The effects of AAPI-a on the activities of SOD, GSH-Px and the levels of MDA in the liver of the aged mice is shown in Table 4. Apparently, a marked increase

Table 4
The effects of AAP I-a from *A. auricula-judae* on the activities of SOD, GSH-Px and levels of MDA in the liver, serum and heart of aged mice.

Group	Dose (mg/kg/day)	Liver			Serum			Heart		
		SOD (U/mg)	GSH-Px (U/mg)	MDA (U/mg)	SOD (U/mL)	GSH-Px (U/mL)	MDA (U/mL)	SOD (U/mg)	GSH-Px (U/mg)	MDA (U/mg)
I		417.25 ± 16.42[b]	1205.23 ± 83.56[b]	4.49 ± 0.11[b]	236.4 ± 16.30[b]	1210.76 ± 72.21[b]	7.24 ± 0.26[b]	12.39 ± 0.43[a]	1157.23 ± 85.78[b]	8.55 ± 0.12[b]
II		325.85 ± 11.56	804.77 ± 58.42	7.39 ± 0.28	154.22 ± 9.10	847.88 ± 57.24	13.23 ± 0.28	11.24 ± 0.32	695.32 ± 51.32	14.46 ± 0.31
III	50	353.74 ± 13.70[a]	830.28 ± 65.01[a]	5.58 ± 0.12[a]	216.51 ± 12.60[b]	1007.35 ± 70.61[a]	12.50 ± 0.42[a]	11.40 ± 0.29	704.84 ± 42.19	9.18 ± 0.25[b]
IV	100	368.89 ± 12.32[b]	1037.23 ± 78.91[b]	5.60 ± 0.23[a]	220.98 ± 13.30[b]	1169.03 ± 88.20[a]	9.39 ± 0.12[b]	11.89 ± 0.43[a]	767.84 ± 44.31[b]	9.15 ± 0.14[b]
V	200	391.12 ± 15.70[b]	855.29 ± 75.10[a]	5.12 ± 0.15[b]	225.03 ± 11.90[b]	1206.6 ± 85.95[b]	8.16 ± 0.30[b]	12.17 ± 0.39[a]	1045.85 ± 78.23[b]	9.28 ± 0.35[b]

Data were expressed as mean ± S.D. ($n = 10$) and compared using a non-directional hypothesis two-tailed *t*-test; $p < 0.05$ was taken as statistically significant. A Duncan's Multiple Range Test determined which sample means differed significantly from one another. Control and aged model mice (I, II) were fed with a standard mice chow for 35 days. Three groups of polysaccharides-treatment mice (III, IV, V) were orally given polysaccharides in a single dose of 50 mg/kg, 100 mg/kg and 150 mg/kg bodyweight once daily, respectively and fed with a standard mice chow for 35 days.

[a] $p < 0.05$ compared with aged model.

[b] $p < 0.01$ compared with aged model.

in MDA and significant decreases ($p < 0.05$) of antioxidant enzymes activity (SOD, GSH-Px) was observed in the livers between the treated Group I (Normal Control Group) and Group II (Model Control Group). The AAP I-a treatment inhibited significantly ($p < 0.05$) the formation of MDA in the mice livers and raised the activity of antioxidant enzymes in a dose-dependent manner (Groups III and IV). The administration of AAPI-a to the D-galactose treated mice (Groups II, III, IV and V) with 100 and 200 mg/kg increased the activity of both SOD and GSH-Px enzymatic antioxidants in both groups livers ($p < 0.01$ and $p < 0.05$).

Free-radical scavenging enzymes such as SOD and GSH-Px are the first line of defense against oxidative injury. They are involved in the reduction of reactive oxygen species (ROS) and peroxides produced in a living organism as well as in the detoxification of certain compounds of exogenous origin and serve as a potential marker of susceptibility, early and reversible tissue damage and a decrease in antioxidant defense (Lv et al., 2007). Lipid peroxidation generates many aldehyde products, among which MDA is considered the most important derivative and has been frequently used as markers of oxidative stress (Urso & Clarkson, 2003). As shown in Table 3, the administration of AAP I-a elevated the activities of antioxidant enzymes (SOD and GSH-Px), while at the same time it reduced the level of MDA in the blood serum (Groups III to V). Therefore, AAP I-a may reduce oxidative stress and ageing phenotypes by increasing SOD and GSH-Px activities and, as well, reduce the level of MDA although these mechanisms remain to be clarified. In this test, probability value ($p < 0.05$) and ($p < 0.01$) was also observed in blood serum treatments of AAP I-a; meaning that the total antioxidant capability of the antioxidant systems in organism had been enhanced.

The effects of AAP I-a on the activity of antioxidant enzymes in the hearts of aged mice are presented in Table 4. To explain the mechanism of purified *A. auricula* polysaccharide (AAP I-a) in improving heart function using the aged laboratory mice model; examined were the antioxidant enzyme activity of each mouse's heart together with the MDA level.

The identified antioxidant enzymes act cooperatively at different sites in the metabolic pathway of free radicals to prevent oxidant damage. A vast amount of evidence implicates that ageing is associated with a decrease in antioxidant status and the age-dependent increase in lipid peroxidation is a consequence of diminished antioxidant protection (see: Schuessel et al., 2006). The age-related decrease in the activity of SOD documented in this study is in agreement with earlier investigations by Wu et al. (2010).

5. Conclusions

The discovery of new drugs from traditional Chinese medicine is not a new phenomenon. The combination of HPLC/GPC, FTIR spectroscopy, and GC–MS methods is useful to characterize the chemical structures of bioactive polysaccharide. To make clear the chemical structures and chain conformations of polysaccharide it is important to understand their biological activities. Therefore, the research findings show AAP I-a to be potent anti-oxidant in preventing free radical reactions in vivo. Furthermore, AAP I-a polysaccharide therapeutic treatment, when consumed for 35 consecutive days, showed no noticeable significant physical change to the examined vital organs or on the body weight of the treated aged mice. This research found that polysaccharide treatment can significantly increase the thymus index and spleen index vitality of the superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and reduce the content of the peroxidation product—malondialdehyde (MDA). It was also shown that SOD and GSH-Px activity decreased markedly with ageing and that these changes had statistical significance in the liver and blood serum.

It is likely that the decrease in the activities of SOD and GSH-Px is the main factor in lipid peroxidative damage. Similarly, the result of polysaccharide effects on enhancing the heart and blood serum antioxidant enzyme activity in mice is presented in Table 3. Blood serum and heart anti-oxidant enzymes (SOD, GSH-Px) activity and the level in model mice (Group II) were significantly decreased ($p < 0.01$) in comparison with the control group mice (Group I). There was a significant difference in blood serum, heart, and liver anti-oxidant enzyme activity between groups after seven weeks of polysaccharide supplementation. In both the Groups III and IV mice, polysaccharide supplementation significantly ($p < 0.01$) enhanced blood serum heart and liver anti-oxidant enzyme activity. It is therefore, concluded that AAPI-a has an anti-ageing effect and a potential therapeutic action in vivo.

Acknowledgements

This research was supported by a grant from the Chinese National High Technology Research and Development Program (863 Program, Grant No. 2007AA100404). The author expresses her thanks to Professor Bob Tuck from Australia who edited and refined the paper.

References

Bagchi, D., Bagchi, M., Hassoun, E. A., & Stohs, S. J. (1995). In-vitro and in-vivo generation of reactive oxygen species DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology*, *104*, 129–140.

Bonnefont, R. D., Bastard, J. P., Jandon, M. C., & Delattre, J. (2000). Consequences of the diabetic status on oxidant/antioxidant balance. *Diabetes and Metabolism*, *26*, 163–176.

Chang, J. S., Kim, H. J., Bae, J. T., Park, S. H., Kim, S. E., Kim, O. M., et al. (1998). Inhibition effects of *A. auricula-judae-judae* methanol extract on lipid peroxidation and liver damage in benzo(α)pyrene-treated mice. *Journal of the Korean Society of Food Science and Nutrition*, *27*, 712–717.

Chen, G., Luo, Y. C., Ji, B. P., Li, B., Guo, Y., Li, Y., et al. (2008). Effect of polysaccharide from *Auricularia auricula* on blood lipid metabolism and lipoprotein lipase activity of ICR mice fed a cholesterol-enriched diet. *Journal of Food Science*, *73*, 103–108.

Ding, L. (1994). The isolation of polysaccharides from *Auricularia auricala* and it's monosaccharide analysis. *Special Wild Economic Animal and Plant Research*, *1*, 44–45.

Fan, L. (2006). Studies on the preparation and bioactivities of the AAP-II a fraction of polysaccharides from *Auricularia auricala*. PhD thesis, Huazhong Agricultural University.

Fan, L. S., Zhang, S. H., Yu, L., & Ma, L. (2006). Evaluation of antioxidant property and quality of breads containing *Auricularia auricula* polysaccharide flour. *Food Chemistry*, *101*, 1158–1163.

Fellah, A., Anjukandi, P., Waterland, M. R., & Williams, M. A. K. (2009). Determining the degree of methylesterification of pectin by ATR/FT-IR: Methodology optimisation and comparison with theoretical calculations. *Carbohydrate Polymers*, *78*, 847–853.

Golden, T. R., & Melov, S. (2001). Mitochondrial DNA mutations, oxidative stress, and ageing. *Mechanisms of Ageing and Development*, *122*, 1577–1589.

He, Y. M., Liu, C. H., Chen, Y. X., Ji, A. C., Shen, Z. L., Xi, T., et al. (2007). Isolation and structural characterization of a novel polysaccharide prepared from Arca subcrenata Lischke. *Journal of Bioscience and Bioengineering*, *104*, 111–116.

Hu, Y., Xu, J., & Hu, Q. H. (2003). Evaluation of antioxidant potential of aloe vera (Aloe. barbadensis Miller) extracts. *Journal of Agricultural and Food Chemistry*, *51*, 7788–7791.

Jiang, Y. H., Jiang, X. L., Wang, P., & Hu, X. K. (2005). In-vitro antioxidant activities of water-soluble polysaccharides extracted from Isaria farinosa B05. *Journal of Food Biochemistry*, *29*, 323–335.

Kardošová, A., & Machová, E. (2006). Antioxidant activity of medicinal plant polysaccharides. *Fitoterapia*, *77*, 367–373.

Ke, C. L., Qiao, D. L., Gan, D., Sun, Y., Ye, H., & Zeng, X. X. (2009). Anti-oxidant activity in-vitro and in-vivo of the capsule polysaccharides from *Streptococcus equi* subsp.zooepidemicus. *Carbohydrate Polymers*, *75*, 677–682.

Ke, R. D., Lin, S. F., Chen, Y., Ji, C. R., & Shu, Q. G. (2010). Analysis of chemical composition of polysaccharides from *Poria cocos* Wolf and its anti-tumor activity by NMR spectroscopy. *Carbohydrate Polymers*, *80*, 31–34.

Koyama, K., Akiba, M., Imaizumi, T., Kinoshita, K., Takahashi, K., Suzuki, A., et al. (2002). Antinociceptive constituents of *Auricularia polytricha*. *Planta Medica*, *68*, 284–285.

Li, X. M., Ma, Y. L., & Liu, X. J. (2007). Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. *Journal of Ethnopharmacology*, *111*, 504–511.

Lin, M. T., & Beal, M. F. (2003). The oxidative damage theory of ageing. *Clinical Neuroscience Research*, *2*, 305–315.

Liu, C. H., Lin, Q. X., Gao, Y., Ye, L., Xing, Y. Y., & Xi, T. (2007). Characterization and anti-tumor activity of a polysaccharide from strongylocentrotus nudus eggs. *Carbohydrate Polymers*, *67*, 313–318.

Liu, C. H., Wang, C. H., Xu, Z. L., & Wang, Y. (2007). Isolation, chemical characterization, and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with seawater. *Process Biochemistry*, *42*, 961–970.

Lu, J., Zheng, Y. L., Wu, D. M., Luo, L., Sun, D. X., & Shan, Q. (2007). Ursolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. *Biochemical Pharmacology*, *74*, 1078–1090.

Luo, Y. C., Chen, G., Li, B., Ji, B. P., Guo, Y., & Tian, F. (2009). Evaluation of anti-oxidative and hypolipidemic properties of a novel functional diet formulation of *Auricularia auricula* and Hawthorn. *Innovative Food Science and Emerging Technologies*, *10*, 215–221.

Lv, L. S., Gu, X. H., Tang, J., & Ho, C. T. (2007). Antioxidant activity of stilbene glycoside from Polygonum multiflorum Thunb in-vivo. *Food Chemistry*, *104*, 1678–1681.

Ma, M., Liu, G. H., Yu, Z. H., Chen, G., & Zhang, X. (2009). Effect of the *Lycium barbarum* polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in-vivo. *Food Chemistry*, *113*, 872–877.

Ma, Z. C., Wang, J. G., & Zhang, L. N. (2008). Structure and chain conformation of β-glucan isolated from *A. auricula-judae*. *Biopolymers*, *89*, 614–622.

Ma, Z. C., Wang, J. G., Zhang, L. N., Zhang, Y. F., & Ding, K. (2010). Evaluation of water-soluble β-D-glucan from *Auricularia auricular-judae* as potential anti-tumor agent. *Carbohydrate Polymers*, *80*, 977–983.

Mao, W. J., Li, H. Y., Li, Y., Zhang, H. J., Qi, X. H., Sun, H. H., et al. (2009). Chemical characteristic and anticoagulant activity of the sulfated polysaccharide isolated from *Monostroma latissimum* (Chlorophyta). *International Journal of Biological Macromolecules*, *44*, 70–74.

Martin, I., & Grotewiel, M. S. (2006). Oxidative damage and age-related functional declines. *Mechanisms of Ageing and Development*, *127*, 411–423.

Mau, J. L., Chao, G. R., & Wu, K. T. (2001). Antioxidant properties of methanolic extracts from several ear mushrooms. *Journal of Agricultural and Food Chemistry*, *49*, 5461–5467.

Mau, J. L., Lin, H. C., & Song, S. F. (2002). Antioxidant properties of several specialty mushroom. *Food Research International*, *35*, 519–526.

Mizuno, T., Ando, M., Sugie, R., Ito, H., Shimura, K., Sumiya, T., et al. (1992). Antitumor activity of some polysaccharides isolated from an edible mushroom, Ningyotake, the fruiting body and the cultured mycelium mycelium of Polyporous confluens. *Bioscience Biotechnology and Biochemistry*, *56*, 34–41.

Pang, Z. J., Chen, Y., & Zhou, M. (2000). Polysaccharide krestin enhances manganese superoxide dismutase activity and mRNA expression in mouse peritoneal macrophages. *The American Journal of Chinese Medicine*, *28*, 331–341.

Popescu, C. M., Singurel, G., Popescu, M. C., Vasile, C., Argyropoulos, D. S., & Willför, S. (2009). Vibrational spectroscopy and X-ray diffraction methods to establish the differences between hardwood and softwood. *Carbohydrate Polymers*, *77*, 851–857.

Qiao, D. L., Ke, C. L., Hu, B., Luo, J. G., Ye, H., Sun, Y., et al. (2009). Antioxidant activities of polysaccharides from *Hyriopsis cumingii*. *Carbohydrate Polymers*, *78*, 199–204.

Ramarahnam, N., Osawa, T., Ochi, H., & Kawaishi, S. (1995). The contribution of plant food antioxidants to human health. *Trends in Food Science and Technology*, *6*, 75–82.

Schuessel, K., Frey, C., Jourdan, C., Keil, U., Weber, C. C., Muller-Spahn, F., et al. (2006). Ageing sensitizes toward ROS formation and lipid peroxidation in PS1M146L transgenic mice. *Free Radical Biology and Medicine*, *40*, 850–862.

Sheu, F., Chien, P. J., Chien, A. L., Chen, Y. F., & Chin, K. L. (2004). Isolation and characterization of an immunomodulatory protein (APP) from the Jew's Ear mushroom *Auricularia polytricha*. *Food Chemistry*, *87*, 593–600.

Sone, Y., Kakuta, M., & Misaki, A. (1978). Isolation and characterization of polysaccharides of "Kikurage" fruit body of *Auricularia auricula-judae*. *Agricultural and Biological Chemistry*, *42*, 417–425.

Song, X., Bao, M., Li, D., & Li, Y. M. (1999). Advanced glycation in D-galactose induced mouse-ageing model. *Mechanisms of Ageing and Development*, *108*, 239–251.

Su, X. Y., Wang, Z. Y., & Liu, J. R. (2009). In-vitro and in-vivo antioxidant activity of *Pinus koraiensis* seed extract containing phenolic compounds. *Food Chemistry*, *117*, 681–686.

Sun, S. W., Yu, H. Q., Zhang, H., Zheng, Y. L., Wang, J. J., & Luo, L. (2007). Quercetin attenuates spontaneous behavior and spatial memory impairment in D-galactose-treated mice by increasing brain antioxidant capacity. *Nutrition Research*, *27*, 169–175.

Sun, Z. W., Zhang, L. X., Zhang, B., & Niu, T. G. (2010). Structural characterisation and antioxidant properties of polysaccharides from the fruiting bodies of *Russula virescens*. *Food Chemistry*, *118*, 675–680.

Tsiapali, E., Whaley, S., Kalbfleisch, J., Ensley, H. E., Browder, W., & Williams, D. L. (2001). Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity. *Free Radical Biology and Medicine*, *30*, 393–402.

Ukai, S., Kiho, T., Hara, C., Morita, M., Goto, A., Imaizumi, N., et al. (1983). Polysaccharides in fungi: XIII. Anti-tumour activity of various polysaccharides isolated from Dictyophora indusiata, Ganoderma japonicum, Cordyceps cicadae. *Auricularia auricula-judae* and Auricularia species. *Chemical & Pharmaceutical Bulletin*, *31*, 741–744.

Urso, M. L., & Clarkson, P. M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, *189*(1/2), 41–54.

Wu, J., Ding, Z. Y., & Zhang, K. C. (2006). Improvement of exopolysaccharide production by macro-fungus *Auricularia auricula* in submerged culture. *Enzyme and Microbial Technology*, *39*, 743–749.

Wu, Q., Tan, Z. P., Liu, H. D., Gao, L., Wu, S. J., Luo, J. W., et al. (2010). Chemical characterization of *Auricularia auricula* polysaccharides and its pharmacological effect on heart anti-oxidant enzyme activities and left ventricle ejection fraction in aged mice. *International Journal of Biological Macromolecules*, *46*, 284–288.

Xia, E. N., & Chen, Q. H. (1988). The separation, purification and identification of polysaccharides from *Auricularia Auricala*. *Acta Biochimica et Biophysica Sinica*, *20*, 614–618.

Yan, P. S., Luo, X. C., & Zhou, Q. (2004). RAPD molecular differentiation of the cultivated strains of the jelly mushrooms. *Auricularia auricula* and *A. polytricha*. *World Journal of Microbiology and Biotechnology*, *20*, 795–799.

Yang, L. Q., & Zhang, L. M. (2009). Chemical structural and chain conformational characterization of some bioactive polysaccharides isolated from natural sources. *Carbohydrate Polymers*, *76*, 349–361.

Yoon, S. J., Yu, M. A., Pyun, Y. R., Hwang, J. K., Chuc, D. C., Junejac, L. R., et al. (2003). The nontoxic mushroom *Auricularia auricula* contains a polysaccharide with anticoagulant activity mediated by anti-thrombin. *Thrombosis Research*, *112*, 151–158.

Zalys, R., Zagon, I. S., Bonneau, R. H., Lang, C. M., & McLaughlin, P. J. (2000). In-vivo effects of chronic treatment with [Met5]-enkephalin on hematological values and natural killer cell activity in athymic mice. *Life Sciences*, *66*, 829–834.

Zhang, L. N., & Yang, L. Q. (1995). Properties of *A. auricula-judae* β-D-glucan in dilute solution. *Biopolymers*, *36*, 695–700.

Zhang, L. N., Yang, L. Q., & Chen, J. H. (1995). Conformational change of the β-D-glucan of *A. auricula-judae* in water-dimethyl sulfoxide mixtures. *Carbohydrate Research*, *276*, 443–447.

Zhang, L. N., Yang, L. Q., Ding, Q., & Chen, X. F. (1995). Studies on molecular weights of polysaccharides of *A. auricula-judae*. *Carbohydrate Research*, *270*, 1–10.

Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Anti-tumor polysaccharides from mushrooms a review on their isolation process, structural characteristics and anti-tumor activity. *Food Science & Technology*, *18*, 4–19.

Zhang, W. J. (1999). *Biochemical technology of carbohydrate complexes*. Hangzhou: Zhejiang University Press.

Zhao, G. H., Kan, J. Q., Li, Z. X., & Chen, Z. D. (2005). Characterization and immunostimulatory activity of an (1 → 6)α-D-glucan from the root of Ipomoea batatas. *International Immunopharmacology*, *5*, 1436–1445.

Zhou, Y. C., & Zheng, R. L. (1991). Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. *Biochemical Pharmacology*, *42*, 1177–1179.