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Structural analysis of water-soluble polysaccharides in the fruiting body of *Dictyophora indusiata* and their *in vivo* antioxidant activities

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ABSTRACT

The water-soluble *Dictyophora indusiata* polysaccharides (DIP) were extracted from the fruiting body of *D. indusiata*. The structural features of purified DIPs I and II were investigated. The results indicated that DIP I was composed of glucose (Glc) and mannose (Man) with molecular weight of 2100 kDa, while DIP II comprised of xylose (Xyl), galactose (Gal), glucose (Glc) and Man with molecular weight of 18.16 kDa. The glycosidic linkage of DIP I was composed of →1)-Glc-(6→: →1)-Man-(3,6→ with the ratio of 5.6:1.0, while DIP II was composed of →1)-Glc-(6→: →1)-Man-(3,6→: →1)-Xyl-(5→: →1)-Gal-(3→: →1)-Gal-(6→: with the ratio of 4.9: 15.5: 7.8: 1.0: 5.7. DIP significantly ($P < 0.05$) decreased the malondialdehyde (MDA), lipofuscin levels and increased the superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities of mice. The strong *in vivo* antioxidant activity indicated DIP had great potential as functional food.

1. Introduction

Mushroom is a fleshy spore-bearing fruiting body of fungus, which is typically generated on soil or on its food source. Mushroom is a low-calorie food and a good source of vitamins. The edible mushroom polysaccharides have strong antioxidant activities and can be explored as novel potential antioxidants in food and pharmaceutical industry (Jiang, Jiang, Wang, & Hu, 2005). Several polysaccharides have been isolated from edible fungi, such as *Morchella esculenta* (Duncan, Pugh, Pasco, & Ross, 2002); *Coriolus versicolor* (Ng, 1998), and *Lentinus edodes* (Leatham & Stahmann, 1981). In recent years, there has been an increasing interest to utilize these polysaccharides as a source of therapeutic agents in medicine and cosmetics (Wu, Sun, & Pan, 2006). The health effects of edible fungi polysaccharides in human diet involve in antioxidation, antitumor, anticancer, immunomodulation and anti-cardiovascular diseases (Ajith & Janardhanan, 2007; Wang, Liu, Ng, Ooi, & Chang, 1995; Wasser, 2002; Zhang, Cui, Cheung, & Wang, 2007). Some polysaccharides have been commercially developed into important components of therapeutic drugs and skin care products (Ajith & Janardhanan, 2007). The structural characteristics of polysaccharides are critical for the health-beneficial capabilities. The galactoglucan of mushroom *Pleurotus florida* blue variant shows great immunoenhancing activity (Dey et al., 2010).

Dictyophora indusiata is an edible mushroom well-accepted by consumers in China and other Asian countries. It is called "queen of the mushrooms" due to the beautiful appearance, delicious taste and health benefit. Some bioactive compounds have been isolated from *D. indusiata* (Lee et al., 2002; Sharma, Choi, Sharma, Choi, & Seo, 2004), such as *N*-methyl-daspartate and *R*-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, 5-(hydroxymethyl)-2-furfural (Sharma et al., 2004). Although studies on antioxidation of polysaccharides from fungi such as *Cordyceps sinensis* and *Agaricus sylvaticus* have been reported (Li & Xu, 1997; Percurio, 2008). Most of work was conducted by antioxidation assay system *in vitro*. It is known that antioxidants showing great antioxidant activity *in vitro* might have a poor antioxidant activity *in vivo*. Therefore, it is significant to test the antioxidant activity of polysaccharide *in vivo*. However, up to now, there is no report on polysaccharides from the fruiting body of *D. indusiata*. It is worthwhile to purify the polysaccharides and to characterize their structural characteristics. These results will be of great significance to further utilize this mushroom resource. Therefore, the objective of this work was to extract water-soluble DIP and to characterize the monosaccharide composition, glycosidic linkage and molecular weight of purified polysaccharide fractions. Moreover, the antioxidant activities of DIP from the fruiting body of *D. indusiata* were also investigated *in vivo* and *in vitro*.

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2. Materials and methods

2.1. Materials

The fruiting body of *D. indusiata* (dried product) were purchased from a local commercial market in Gutian, China. Glucose, phenol and sulfuric acid were obtained from Shanghai Reagent Co. (Shanghai, China). Standards of xylose (Xyl), arabinose (Ara), glucose (Glc), galactose (Gal), fructose (Fru), fucose (Fuc), mannose (Man), galacturonic acid (GalA), glucuronic acid (GlcA) and dextrans of different molecular weights were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Assay kits for malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were obtained from Nanjing Jiancheng Bioengineering Institute. Vitamin E capsule (yellow, batch: HE30038) was purchased from Qunxing Co. Ltd. (China). Fluorescein disodium (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azobis(2-methylpropionamide)-dihydrochloride (AAPH) were also purchased from Sigma Chemical Co (St. Louis, MO, USA). All the other chemicals used were of analytical grade.

2.2. Preparation of DIP

The fruiting body of *D. indusiata* was pulverized by a miller. The powder was went through a 60-mesh screen. They (500 g) were precisely weighted and then extracted with 15,000 ml of distilled water at 100 °C for 2 h. After centrifuge at 3000 × *g* for 15 min, the supernatant was collected and the residues were subjected to above extraction for two times. The combined supernatants were concentrated to one tenth of the volume by rotary vacuum evaporator (BC-R203, Shanghai Biochemical Equipment Co., Shanghai, China) at 65 °C, and then deproteinized with Sevag regent ($CHCl_3$:*n*-BuOH = 4:1, v/v). The polysaccharide solution was precipitated by stepwise addition of ethanol to concentrations of 30% and 80%. Each precipitate was collected by centrifugation at 3000 × *g* for 15 min. Then they were lyophilized for further analysis. The polysaccharide fraction precipitated by 80% ethanol was termed DIP, which was used for further separation and animal test.

2.3. Separation and purification of DIP

Fifty milligrams of DIPs dissolved in 100 ml of distilled water was applied to a Sephadex G-75 column (5 cm × 50 cm, GE, Shanghai, China) pre-equilibrated with water. They were eluted with distilled water until no carbohydrate was detected. Each fraction was assayed for carbohydrate content by phenol–sulfuric acid method. The major fraction was further purified with DEAE-Sepharose fast flow column (5 cm × 20 cm, GE, Shanghai, China). It was eluted with distilled water firstly, then with NaCl gradient (0.1, 0.2, 0.3, 0.5 and 1 M) until no carbohydrate is detected. The fractions eluted by distilled water and 0.1 M NaCl (DIPs I and II) were dialyzed and lyophilized.

2.4. Molecular weight determination of DIPs I and II

The molecular weight was evaluated by gel-permeation chromatography according to the method of Han et al. (2011) on a Waters instrument (Waters Co., USA) with a TSKGel G4000SWXL column. Waters 2410 refractive index detector (MA, USA) was used for determination. The standard dextrans (molecular weights of 2000, 188, 76.9, and 10.5 kDa) were used for the calibration curve, and then the retention times were plotted against the logarithms of the corresponding average molecular weights. Mobile phase: 0.02 M KH_2PO_4 buffer (pH 6.0), flow rate of 0.6 ml/min, temperature of 40 °C, injection volume of 20 μL. The retention time of the purified TFP fractions (1 mg/ml) were then plotted in the same graph, and the average molecular weight was determined.

2.5. Analysis of monosaccharide composition

DIP I or II (10 mg) were hydrolyzed by 10 ml of 2 M trifluoroacetic acid at 100 °C for 2 h (Erbing, Jansson, Widmalm, & Nimmich, 1995). Derivatization of the released monosaccharides was then carried out by the trimethylsilylation reagent according to the method of Guentas et al. (2001). The trimethylsilylated derivatives were determined by gas chromatography. The following programme was adopted for gas chromatography analysis with an RTX-5 fused silica capillary column (30 m × 0.32 mm, film thickness 0.25 μm; Amchro, SulzbachPTaunus, Germany) (Yang et al., 2009): injection temperature: 230 °C; detector temperature: 230 °C; column temperature programmed from 130 to 180 °C at 2 °C/min, holding for 3 min at 180 °C, then increasing to 220 °C at 10 °C/min and finally holding for 3 min at 220 °C. The injection was in spiltless mode. Inositol was used as the internal standard.

2.6. Methylation analysis

Methylation of DIP I (or DIP II) was carried out according to the method of Jiang et al. (2009) with minor modification. Ten milligrams of dried DIP I were weighted precisely and dissolved in 8.0 ml of DMSO. The mixture was then treated by ultrasonic wave for 15 min. After incubation for 30 min at room temperature (25 °C), 2 ml of methyl iodide were added for methylation. The sample was kept in dark for 24 h before 4.0 ml of distilled water were used to decompose the remained methyl iodide. The methylated polysaccharides were extracted by 4 ml × 3 ml of chloroform and dried at low pressure by a rotary evaporator (RE52AA, Yarong Instrument Co., Shanghai, China) at 60 °C. After hydrolysis by 10 ml of 2 M trifluoacetic acid at 100 °C for 2 h, the fluid was dried as above. Twenty milligrams of $NaBH_4$ were added for reduction. Glacial acetic acid was used to terminate the reduction. The sample was dried under low pressure, and then acetylated by 2 ml of acetic anhydride and 2 ml of pyridine. The reaction was kept at 100 °C for 1 h. Distilled water was used to decompose the remained acetic anhydride. The acetylated derivatives were extracted by 4 ml of methylene chloride. A gas chromatography/mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyze the glycosidic linkage. The acetylated derivatives were loaded into a RTX-5 fused silica capillary (30 m × 0.32 mm, film thickness 0.25 μm; Amchro, SulzbachPTaunus, Germany). The temperature program was set as follows: the initial temperature of column was 150 °C, increased to 260 °C at 10 °C/min, held for 5 min at 260 °C; injection temperature: 260 °C. The ion source of mass spectrometer was set at 280 °C. One microliter of sample was injected and the split ratio was 50:1.

2.7. Determination of vivo and vitro antioxidant activity of DIPs

2.7.1. Animals preparation and experiment design

Sprague Dawley (SP) rats of SPF-level (365 days old, weighing 220–250 g, male) were provided by the Guangdong Medical Experimental Animal Center. The rats were kept for a week under controlled conditions of 12 h/12 h light–dark cycle and 50% relative humidity at 25–30 °C. They were also free access to standard food and water. Animals were handled according to the rules and regulations of Institutional Animal Ethics Committee (IAEC), China Medical University, China.

The rats were randomized to six groups (12 animals per group): normal control group, model control group, vitamin C (25 mg/kg body weight) group (positive control), and three treatment groups as follows: low-dose DIPs with 2.7 ml/kg/d, medium-dose DIPs with 5.4 ml/kg/d, high-dose DIPs with 16.2 ml/kg/d. DIP (1.6 mg/ml)

in distilled water was used. Except the normal control group, each group was induced by a single intraperitoneal injection of D-galactose (200 mg/kg/d body weight, dissolved in a 0.9% saline solution) for 42 days (Lv, Gu, Tang, & Ho, 2007). The rats in the normal control group were given the same volume of physiological saline solution (0.9%, w/v) once daily for 6 weeks by intraperitoneal injection. Rats in the three treatment groups were intragastrically administered for 42 days (once a day). The rats in the normal control group and model control group were gastric fed with the same volume of distilled water for a period of 42 days. One hour after the last drug administration, the rats were weighed, then rats were killed by cervical dislocation and blood samples were obtained from the eyepit of the rats and processed for serum (1467 × *g* for 10 min at 4 °C). The brain, liver, heart, spleen, kidney, adrenal grand, prostate, spermary and thymus were removed and washed thoroughly with ice-cold saline. After weighting, these tissue indexes were calculated according to the following formula:

$$\text{One tissue index}\,(\%) = \frac{\text{The tissue weight}}{\text{Animal body weight}} \times 100$$

2.7.2. Assays of SOD, GSH-Px and MDA in rat serums

The superoxide dismutase (SOD) activity, the malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) level were also measured according to the instructions on the kits.

2.7.3. Assays of lipofuscin level in rats livers

The lipofuscin level was determined by previous method with slight modifications (Shen, Shang, & Li, 2011). Briefly, 2 ml of 10% (w/v) liver tissue homogenate was mixed into 4 ml of chloroform–methanol solution (2:1, v/v). After centrifugation at 820 × *g* for 10 min, the lipofuscin levels were evaluated in the chloroform phase using a RF-510 fluorescence spectrophotometer (Shimadzu, Japan). The luminescence of the sample was determined at an emission maximum of 450 nm. The concentration of lipofuscin was determined using quinine sulfate (0.1 g/ml) as a standard.

2.7.4. Antioxidant activity of DIPs by ORAC

The oxygen radical absorbance capacity (ORAC) was measured by ORAC assay of Hernández-Ledesma, Amigo, Recio, and Bartolom (2007) with minor modifications. AAPH and Trolox solutions were prepared freshly and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4). Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4), and the final assay mixture (200 μl) contained 20 μl of phosphate buffer, 20 μl of fluorescein (70 nM), AAPH of 140 μl (12.8 mM), and 20 μl of antioxidant [Trolox (1–8 μM) or sample (at different concentrations)]. A Polarstar Galaxy plate reader (ThermoFisher Scientific, USA) with 485-Pexcitation and 528-Pemission filters was used. The equipment was controlled by the SkanIt software version (2.4.3) for fluorescence measurement. Black 96-well microplates were used. The plate was automatically shaken before the first reading, and the fluorescence was recorded every 2 min for 108 min.

All the reaction mixtures were prepared in duplicate and at least three independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (no antioxidant). From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as $\text{AUC} = 1 + \sum_{i=1}^{i=54} f_i/f_0$ where f_0 is the initial fluorescence at 0 min and f_i is the fluorescence at time $i/2$. The net AUC corresponding to a sample was calculated as net $\text{AUC} = \text{AUC}_{\text{antioxidant}} - \text{AUC}_{\text{blank}}$.

The regression equation between net AUC and antioxidant concentration was calculated. The slope of the equation was used to calculate the ORAC value by the Trolox curve obtained for each assay. Final ORAC values were expressed as μmol of Trolox equivalent (TE)/μmol of antioxidant. Analysis were carried out in triplicate.

Table 1
Monosaccharide composition (relatively molar percentage) and glycosidic linkage of DIPs I and II.

Compositions	DIP I	DIP II
Glc	86.3%	13.9%
Man	13.7%	44.9%
Xyl	–	22.6%
Gal	–	18.6%
Molar ratio of glycosidic linkage		
→1)-Glc-(6→	5.6	4.9
→1)-Man-(3,6→	1.0	–
→1)-Man-(2→	–	15.5
→1)-Xyl-(5→	–	7.8
→1)-Gal-(3→	–	1.0
→1)-Gal-(6→	–	5.7

2.8. Statistical analyses

Data were expressed as mean ± standard deviation of three replicated determinations. One way of variance analysis was applied for determining significant difference at $P < 0.05$ using SPSS 11 (SPSS Inc., Chicago, USA).

3. Results and discussion

3.1. Characterization of polysaccharide fractions DIPs I and II

DIP I (II) was hydrolyzed by trifluoracetic acid into individual monosaccharides that were further trimethylsilylated for monosaccharide identification. Through comparing the retention time with standards, the monosaccharide composition was identified (Table 1). Three monosaccharides, including Glc and Man, were identified for DIP I. DIP II was consisted of Xyl, Gal, Glc and Man. The relatively molar percentages of Glc, Man in DIP I were 86.3%, 13.7%, while 13.9%, 44.9% for DIP II, and the relatively molar percentages of Xyl and Gal were 22.6% and 18.6%. The results suggested that Glc and Man constructed the backbone for DIPs I and II, respectively. Table 1 shows the glycosidic linkages of DIPs I and II determined by GC/MS analysis. The glycosidic linkages of DIP I were not in coincidence with those of DIP II. In polysaccharide fraction of DIPs I and II, Glc was detected as →1)-Glc-(6→. Man was detected as →1)-Man-(3,6→ in DIP I, but in DIP II as →1)-Man-(2→. Gal was detected as →1)-Gla-(3→ and →1)-Gla-(6→ in DIP II. However, the molar ratio of each linkage was also different between DIPs I and II.

High performance gel permeation chromatography was often employed to determine the molecular weight of polysaccharide. The molecular weights of DIPs I and II were estimated to be 2100.99, and 18.16 kDa, respectively. The polysaccharide fractions DIP I or DIP II appeared no absorption at 260 and 280 nm in the UV spectrum, indicating the absence of nucleic acid and protein conjugate with polysaccharide (data were not shown).

3.2. Antioxidant activity of DIP in vivo

3.2.1. Effect of the DIP on body weight and different indice in aging rats

The effect of DIP on body weight and different indice in aging rats is shown in Table 2. Compared with the normal control group, the thymus index of the model control group was significantly decreased ($P < 0.05$), whereas body weight of rats and brain, heart, liver, kidney, adrenal grand, prostate and spermary indice showed no significant change ($P > 0.05$). D-Galactose can accelerate

Table 2
Effect of DIP on body weight and different organs indice in aging rats.

Group	Body weight (g)	Brain index (%)	Heart index (%)	Liver index (%)	Spleen index (%)
Normal control	444.6 ± 28.2	0.448 ± 0.038	0.321 ± 0.031	3.367 ± 0.425	0.155 ± 0.026
Model control	437.6 ± 29.2	0.446 ± 0.033	0.328 ± 0.049	3.242 ± 0.314	0.162 ± 0.025
DIP low-dose	437.4 ± 52.6	0.471 ± 0.048	0.320 ± 0.049	3.233 ± 0.441	0.163 ± 0.027
DIP medium-dose	443.2 ± 26.8	0.449 ± 0.029	0.337 ± 0.045	3.066 ± 0.549	0.173 ± 0.015
DIP high-dose	425.9 ± 26.4	0.469 ± 0.036	0.317 ± 0.039	3.013 ± 0.443	0.160 ± 0.019
Positive control	433.1 ± 34.9	0.451 ± 0.028	0.319 ± 0.049	3.152 ± 0.279	0.161 ± 0.024

Group (%)	Kidney index	Adrenal grand index (mg%)	Prostate index (%)	Spermary index (%)	Thymus index (%)
Normal control	0.288 ± 0.051	11.5 ± 2.5	0.154 ± 0.035	0.436 ± 0.042	0.108 ± 0.018*
Model control	0.298 ± 0.032	10.5 ± 3.1	0.148 ± 0.037	0.435 ± 0.022	0.078 ± 0.019
DIP low-dose	0.294 ± 0.022	11.5 ± 2.2	0.141 ± 0.026	0.431 ± 0.037	0.088 ± 0.019
DIP medium-dose	0.287 ± 0.024	10.6 ± 3.4	0.148 ± 0.018	0.429 ± 0.028	0.091 ± 0.023
DIP high-dose	0.300 ± 0.025	11.3 ± 2.5	0.145 ± 0.025	0.433 ± 0.043	0.096 ± 0.028
Positive control	0.290 ± 0.019	11.0 ± 2.7	0.148 ± 0.036	0.442 ± 0.044	0.099 ± 0.026*

* Indicates the result is significantly ($p < 0.05$) different to the model control.

Table 3
Effects of DIP on the activity of MDA, SOD and GSH-Px *in serum* of aging rats.*

Group	Dose (ml/kg)	MDA (nmol/ml)	SOD (U/ml)	GSH-Px (U)
Normal control	–	4.79 ± 0.55a	225.9 ± 8.0d	173.0 ± 15.8b
Model control	–	6.01 ± 0.49d	200.2 ± 4.5a	131.5 ± 26.4a
DIP low-dose	2.7	5.57 ± 0.52 cd	217.2 ± 11.3c	156.3 ± 25.7b
DIP medium-dose	5.4	5.41 ± 0.64bc	219.0 ± 9.2 cd	162.6 ± 21.4b
DIP high-dose	16.2	5.04 ± 0.56ab	223.9 ± 4.7 cd	168.4 ± 23.7b
Positive control	25	4.94 ± 0.66ab	209.6 ± 10.1b	165.0 ± 28.1b

* The results having the same letter in each column are not significantly ($p > 0.05$) different.

senescence in rodent such as mice and rats (Ho, Liu, & Wu, 2003; Su & Xiangli, 2008; Wei et al., 2005). Although the mechanism of this animal model is not clear, there is a general opinion that galactitol is formed by reduction of galactose after metabolism of the cell. Galactitol cannot be decomposed, and accumulated in the cell to affect the osmosis stress, which results in the swelling of the cell. Finally, it causes the metabolism of sugar and reactive oxygen species in disorder (Ho et al., 2003). After injecting D-galactose continuously for 6 weeks, metabolism of rats was in disorder, and thymus shrinked as a result of diminishing the function of organs, which was in coincidence with the symptom of senescence. Body weight and other indice in the treating groups were not significant ($P > 0.05$) different to the model group. However, thymus index of rats in the positive group (treated with Ve) was increased significantly ($P < 0.05$) compared to the model group.

3.2.2. The effects of DIP on the MDA level, SOD and GSH-Px activities in serums of aging rats

The effects of DIP on the MDA level, SOD and GSH-Px activities in serums in aging rats are shown in Table 3. Compared with the normal control group, the MDA level of the model control group was significantly increased ($P < 0.05$), while the SOD and GSH-Px activities were significantly decreased ($P < 0.05$). Table 3 exhibits a significant pattern of a decreasing MDA level and an increasing SOD and GSH-Px activities in blood serum with increasing DIP doses. MDA level in the treatment groups of medium and high doses of DIP had significant differences to the model group ($P < 0.05$). SOD activity in treatment groups of low, medium, high doses of DIP also had significant differences to the model group ($P < 0.05$), while GSH-Px activity was similar to SOD activity, which was increased significantly at three doses. The test also showed that the positive group of Ve could improve the MDA level, SOD and GSH-Px activities.

MDA levels in all the organs were significantly increased with aging. MDA is a main marker of endogenous lipid peroxidation. The increase of MDA production indicates increased peroxidative damage with the aging process (Zhang et al., 2003). DIP could successful inhibit lipid peroxidation as observed in the reduction of MDA production in aging rats. The possible mechanism of action is direct protection of membranes from oxygen radical damage (Amagase, Sun, & Borek, 2009). Inal, Kanbak, and Sunal (2001) have reported that erythrocyte MDA levels are increased with the increase of age in healthy subjects. It is in consistent with the results of this work. In animal model injected by D-galactose, the increased production of free radicals and decreased activity of antioxidant enzymes, especially SOD, can be observed (Inal et al., 2001). The results of this work indicated DIP could induce an enhancement of SOD and GSH-Px activities and a scavenging effect on MDA *in vivo*.

3.2.3. Effect of DIP on the lipofuscin level in liver tissue of aging rats

Lipofuscin content is increased with the increase of animal age. Thus, it is regarded as an important index of body aging (Sharma, Maurya, & Singh, 1993). Compared with the normal control group, there was a significant ($P < 0.05$) increase of lipofuscin level in the model group (Table 4). The results showed that treatment group with DIP at medium and high doses both significantly ($P < 0.05$) reduced lipofuscin level in rats liver tissue compared with the model group. Moreover, lipofuscin level of Ve positive group were significantly ($P < 0.05$) lower than that of model control group. This result indicated DIP extracts had good antioxidant activity *in vivo*.

Table 4
Effect of DIP on Lipofuscin level in liver of aging rats.*

Group	Dose (ml/kg)	Lipofuscin level (nmol/ml)
Normal control	–	8.17 ± 0.51a
Model control	–	9.49 ± 0.86c
DIP low-dose	2.7	8.95 ± 0.75bc
DIP medium-dose	5.4	8.57 ± 0.63ab
DIP high-dose	16.2	8.41 ± 0.69ab
Positive control	26.8	8.75 ± 0.79ab

* The results having the same letter in each column are not significantly ($p > 0.05$) different.

3.3. Antioxidant activities of DIP extracts in vitro

Oxygen radical absorbance capacity (ORAC) assay was developed by Cao and Prior (1999) in order to quantify the antioxidant capacity of foods. This assay is based on the chemical damage to β-PE caused by a peroxyl radical producing compound (AAPH), reducing the fluorescence emission of β-PE. The presence of antioxidants in the medium can recover the damage and prolong the reduction in the fluorescence emission. The antioxidant capacity of DIP was calculated to be 115.77 μmol TE/mg, which further confirm the good antioxidant activity of DIP. As Man and Glc constructed the backbone of DIP, it can be called as mannoglucan. This polysaccharide has been reported to have other bioactivities. The mannoglucan from *Microellobosporia grisea* exhibited a high antitumor activity (Inoue, Kohno, & Kadoya, 1983). The glucan backbone with Man branches also showed strong antitumor activity against S-180 cancer *in vivo* (Wu, Hu, Pan, Zhou, & Zhou, 2007).

4. Conclusions

The molecular weights of DIPs I and II were estimated to be 2100.99 and 18.16 kDa, respectively. Two monosaccharides, including Glc and Man, were identified for DIP I. DIP II was consisted of Xyl, Gal, Glc and Man. DIP exhibited good antioxidant capacities *in vivo* assays. It can be used in compensating the decline in the activities of antioxidant enzymes (SOD and GSH-Px) and decreasing the MDA level in blood serum and lipofuscin level in liver tissue.

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