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ORIGINAL ARTICLE

Screening and Characterization of an Acid Polysaccharide with Antioxidant Activity *in vitro* and *in vivo* from *Dendrobium aurantiacum* var. Denneanum (Kerr)

Yijun Fan, Xueyan Tan, Juan Zhao, Kaigang Yang, Shengdi Wu, Jiao Zhang, Shangrao Pu, Gang Wang[1], Aoxue Luo

Department of Landscape Plants, Sichuan Agricultural University, [1]Department of Medicinal Plant, Sichuan Agricultural University, Chengdu, China

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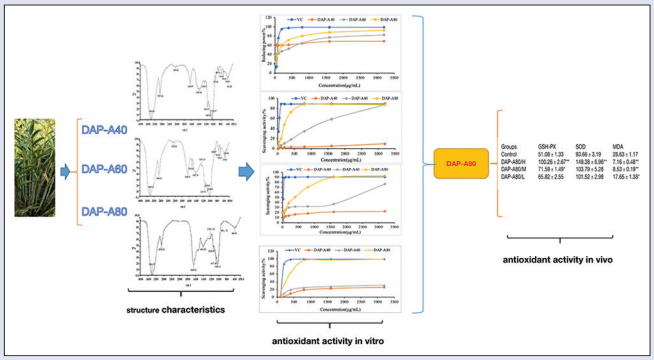
ABSTRACT

Aim: We aimed to monitor an acid polysaccharide with antioxidant activity. **Materials and Methods:** Three acidic polysaccharides (DAP-A40, DAP-A60, and DAP-A80) were extracted from *D. aurantiacum* imbricate by alkaline aqueous solution extraction and ethanol precipitation. The structure characterization and antioxidant activity *in vitro* and *in vivo* were examined. **Results:** The molecular weight (M_W) of three polysaccharides was 1.70 mol[5] Da, 5.78 mol[4] Da, and 1.36 mol[4] Da, respectively, by high-performance gel chromatography. The consequences of infrared spectrum displayed that three polysaccharides enclosed uronic acid and sulfuric acid groups. DAP-A40 and DAP-A60 were collected of α-configuration pyranose. DAP-A80 was composed of β-configuration pyranose. Antioxidant test exhibited that DAP-A80 had outstanding antioxidant activity. The half-maximal inhibitory concentration of DAP-A80 in dipping power and scavenging the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), DPPH, and OH radicals was 163.5 μg/mL, 613.2 μg/mL, 583.2 μg/mL, and 365.1 μg/mL, respectively. The grades of correlation analysis showed that the ability of DAP-A80 to scavenge ABTS, DPPH, and OH radicals was negatively connected with the M_W of polysaccharides and certainly correlated with the content of sulfate group. The results of *in vivo* experiments exposed that DAP-A80 might knowingly decline the formation of malondialdehyde. High levels of DAP-A80 could evocatively upsurge the activity of superoxide dismutase and GSH-Px in mouse serum. **Conclusion:** DAP-A80 has an antioxidant effect and can be reconnoitered as a health medicine and food.

Key words: Acid polysaccharide, antioxidant enzymes, *Dendrobium aurantiacum* var. denneanum (Kerr), free radicals, structure–activity relationship

SUMMARY

- *Dendrobium aurantiacum* var. denneanum (Kerr) are a prized medicinal plant, which has an extensive history in China as Chinese herbal medicine, which is opulent in polysaccharides with strong biological activities. The activities of polysaccharides with different molecular weights and different structural characteristics are unalike. In order to screen and characterize an acid polysaccharide with antioxidant activity, *D. aurantiacum* var. denneanum (Kerr) polysaccharides were prepared by alkaline water extraction and ethanol precipitation and their structural characteristics were investigated. The results exhibited that a new acid polysaccharide DAP-A80 (Mn = 1.36 × 10^4 Da) composed of β-configuration pyranose exhibited a strong ability of scavenging free radicals and can meaningfully surge the activity of superoxide dismutase and GSH-Px in mouse serum. The antioxidant effects were positively allied with the content of sulfate group.



Abbreviations used: DAP: Polysaccharide from *D. aurantiacum* var. denneanum (Kerr); IR: Infrared spectra; OH•: Hydroxyl radicals; ABTS+: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); CAT: Catalase; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; GPC: High-performance Gel Permeation Chromatography; M_W: Molecular weight. Vc: Vitamin C; MDA: Malondialdehyde.

Correspondence:

Dr. Aoxue Luo,
Department of Landscape Plants, Sichuan Agricultural University, Chengdu 611130, China.
E-mail: aoxueluo@sina.com
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INTRODUCTION

Dendrobium is a valuable medicinal plant, which has an extensive history in China as Chinese herbal medicine, tea, and health food. There are 76 species and 2 varieties of *Dendrobium* in China, more than 50 of which can be employed as "Shihu," a popular tonic and a traditional Chinese medicine.[1] Sichuan Province is one of the key producing areas of *Dendrobium*. There are 11 species of *Dendrobium*, among which *D. aurantiacum* var. denneanum (Kerr) is the most copious. It has been originated that *Dendrobium* plants are rich in polysaccharides and have strong biological activities, such as antioxidant and antiaging,[2] hypoglycemic,[3] antitumor,[4,5] and immune regulation.[6,7] At present, the research and application on Dendrobium is mainly dedicated on these species of Dendrobium officinale, Dendrobium huoshanense, and Dendrobium nobile Lindl.[8] Most of these *Dendrobium* species

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grow sluggishly and have low reproductive coefficient, so they want to find germplasm resources through tissue culture. Different from these *Dendrobium* species, *D. aurantiacum* var. denneanum (Kerr), which is also employed as medicine, can attain high reproduction coefficient, vigorous growth, high yield, and rich resources. However, there were few studies on polysaccharides of *D. aurantiacum* var. denneanum (Kerr). Our earlier study found that the content of polysaccharide from *D. aurantiacum* var. denneanum (Kerr) is high,[9] and it can pointedly surge the spleen index and thymus index of mice with S180 sarcoma, promote and regulate the endocrine of interleukin-2 and tumor necrosis factor-α, and coordinate the improvement of immune system function.[10] Therefore, it is of inestimable economic value and great implication to study the active polysaccharides of *D. aurantiacum* var. denneanum (Kerr).

Adverse stimulation can cause oxidative stress and produce a large number of free radicals. If it is not detached in time, it will harm biological molecules such as protein, DNA, and lipid and cause peroxidation reaction. The unsaturated fatty acids in cell membrane are oxidized and destruct the cell structure. At the same time, toxic substances such as hydroperoxide and malondialdehyde (MDA) will be produced, which will delay the normal metabolic activities of human body and lead to analogous diseases disease and accelerated aging process.[11,12] Therefore, the search for competent and low toxic free radical scavengers has become a hot spot in biochemistry and medicine. While plant polysaccharides can lessen oxidative damage and its anti-radiation, anti-inflammatory, anti-fatigue, and antiaging effects are linked to their antioxidant properties. Moreover, the source of polysaccharides is ironic, the extraction is simple, and the side effects are low. Therefore, the antioxidant activity of polysaccharides has progressively attracted people's courtesy.[13,14] In addition to scavenging free radicals directly as hydrogen proton or electron donor, polysaccharides can also act as signal substances to bind to cell receptors and activate intracellular antioxidant-linked signaling pathways. Activation of related transcription pathways can tempt the expression of pro-inflammatory cytokines, antioxidant enzymes, and oxidase genes, thus utilizing immune activity and antioxidant effect.[15] Therefore, in this study, in order to screen and characterize an acid polysaccharide with antioxidant activity, *D. aurantiacum* var. denneanum (Kerr) polysaccharides were arranged by alkaline water extraction and ethanol precipitation and their structural characteristics were scrutinized. Meanwhile, the antioxidant capacity was verified by *in vitro* and *in vivo* experiments.

MATERIALS AND METHODS

Chemical reagent

Superoxide dismutase (SOD), MDA, glutathione peroxidase (GSH-Px), and hydroxyl radical (–OH) test kit were procured from Nanjing Jiancheng Co. (Nanjing, China). Dextrans (2500-2 000 000 Da) were acquired from Pharmacia Co. (Uppsala, Sweden). 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical and Vitamin C (Vc) were obtained from Sigma (St. Louis, USA). ABTS was obtained from Merck(Darmstadt, Germany). Ethanol, petroleum ether, pyridine, hydroxylamine hydrochloride, and all other chemicals and reagents were of analytical grade.

Plant material

The plants were composed in Xiema Township (Sichuan Province, China). They were recognized as *D. aurantiacum* var. denneanum (Kerr) by Professor Aoxue Luo from Sichuan Agricultural University. The stems of *Dendrobium* were unruffled, dried, crushed, and filtered through 40 mother sieves.

Preparation of polysaccharide

Accurately weigh 20.0 g of *D. aurantiacum* var. denneanum (Kerr) powder into a round bottom flask, add 100 mL petroleum ether (60°C–90°C), and extract for 1 h with micro boiling reflux. Fat-soluble substances were removed by filtration. Add 300 mL 0.2 M sodium hydroxide to the dried residue, stir, and extract it for 6 h at room temperature. Collect the filtrate, extract it once again, and combine the filtrate twice. Adjust the filtrate to neutral with hydrochloric acid, extract the protein with Sevage reagent (n-butanol: chloroform = 1:4), and collect the supernatant. Extract for four times, combine the supernatant collected for four times, and rotate to concentrate to 100 mL. Ethanol was added to the polysaccharide solution slowly until the volume of ethanol reached 40%. The precipitate was composed by centrifugation and labeled as DAP-A40. Then absolute ethanol was added to the filtrate to make the volume of ethanol reach 60%. The precipitate was collected and labeled as DAP-A60. Finally, absolute ethanol was added to the filtrate to make the volume of ethanol reach 80%. The precipitate was collected and labeled as DAP-A80. The precipitates were eroded with acetone, ethyl acetate, and anhydrous ethanol and centrifuged, respectively.

Next, the centrifuged precipitate was dissolved with deionized water and put into a dialysis bag (7000 Da). After dialysis (magnetic stirring) with deionized water for 72 h, the water was changed every 8 h. After collecting the dialyzed solution, DAP-A40, DAP-A60, and DAP-A80 were attained by drying.

Determination of uronic acid and sulfate sugar content

According to the method from Ludowieg and Dorfman[16] and Boeley,[17] the standard curve was drawn with dry glucuronic acid as control and the content of uronic acid was measured by sulfuric acid carbazole method. Take 1 mL of polysaccharide solution, add 6 mL concentrated sulfuric acid to each tube in ice water bath, shake well, and then water bath at 85°C for 20 min. After taking out, cool to room temperature, add 0.2 mL 0.1% carbazole solution to each tube, and keep at room temperature for 2 h. Adjust to zero with distilled water, determine the absorbance value with ultraviolet (UV) spectrophotometer at 530 nm wavelength, and calculate the content of uronic acid in each sample. For the determination of the content of sugar sulfate in polysaccharide samples, refer to the method of Dodgson and Price[18] and calculate the content of sugar sulfate by barium chloride gelatin turbidimetric method.

Molecular weight detection

The polysaccharide sample was dissolved in deionized water, and the concentration was 5 mg/mL. The molecular weight (M_W) of each polysaccharide sample was distinguished by HPLC gel permeation chromatography.[19] The recognition conditions were as follows: the column was Waters ultra-hydraulic linear column (ID: 300 mm × 7.8 mm), the column temperature was room temperature and eluted with 0.2 m phosphoric acid buffer, the flow rate was 0.70 mL/min, and the standard reference substance was dextran (2500-2 000 000 Da).

Infrared spectrum analysis

2.0 mg polysaccharide was mixed with KBr, pressed and scanned by Fourier transform infrared spectrometer (scanning range: 4000/cm-500/cm).[20]

Antioxidants *in vitro*

Reducibility determination

The reducibility test of polysaccharide samples was somewhat altered according to the method of Luo *et al*.[21] The samples were prepared with deionized water into solutions of 3200 μg/mL, 1600 μg/mL, 800 μg/mL, 400 μg/mL, 200 μg/mL, 100 μg/mL, 50 μg/mL and 5 μg/mL. Measure 0.5 mL Vc standard solution (positive control), deionized water (blank control), and polysaccharide sample solutions into the test tube and then add 2.5 mL phosphate buffer solution (PBS) (pH 6.6, 0.2 M) to each test tube and shake well. Then add 2.5 mL 30 mM potassium ferricyanide solution to each test tube and shake well. The test tubes were positioned in a water bath at 50°C for 20 min and then returned to normal temperature after standing at room temperature. Then add 2.5 mL trichloroacetic acid solution (0.6 M) to each test tube, shake well, and stand still. Remove 2.5 mL supernatant from each tube and put it into the new test tube. Add 2.5 mL deionized water to the new test tube, shake well, and then add 0.5 mL ferric chloride solution (6 mM) into the new test tube and shake it calmly. The absorbance of each reaction solution at 700 nm was measured by UV spectrophotometer. Three repetitions and calculate the reduction capacity according to the following formula:

$$\text{Clearance rate \%} = \frac{\text{OD (blank control)} - \text{OD(sample)}}{\text{OD (blank control)}} \times 100\%$$

The Ability of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging

0.0192 g ABTS powder was exactly weighed and put into the centrifuge tube. 5 mL 0.01 mol/L PBS solution was added into the tube to dissolve it. After the ABTS powder is entirely dissolved, accurately weigh 0.0033 g potassium persulfate into the centrifugal tube and vibrate to make it fully thawed. Then sealing the centrifuge tube, the tube was placed in the dark at room temperature for 16 h. ABTS radical was formed by reaction. Take appropriate amount of reaction solution, add 0.01 mol/L PBS solution for dilution, so that the absorbance value of the diluted solution at 734 nm is 0.70 ± 0.02. The diluent was then equilibrated at room temperature for 30 min and the absorbance of the solution was determined at 734 nm.[22] 1.0 mL Vc standard solution (positive control), deionized water (blank control), and each polysaccharide sample solution were surely transferred into the test tube. Add 1.0 mL of ABTS solution (A_{734} = 0.70 ± 0.02) to each tube, shake well, and react in the dark for 20 min at room temperature. The absorbance of the solution at 734 nm was measured by UV spectrophotometer. Three repetitions. The clearance rate was calculated as follows:

$$\text{Clearance rate \%} = \frac{\text{OD(blank control)} - \text{OD(sample)}}{\text{OD(blank control)}} \times 100\%$$

DPPH radical scavenging ability test

Specifically transfer 0.3 mL Vc standard solution (positive control), deionized water (blank control), and polysaccharide sample solutions of various concentrations into the test tube, add 0.9 mL DPPH solution (0.1 mmol/L), respectively, and react in the dark for 30 min at room temperature. Then, the absorbance of the reaction solution at 517 nm was determined by UV spectrophotometer. Three repetitions.[23] The clearance rate is calculated as follows:

$$\text{Clearance rate \%} = \frac{\text{OD(blank control)} - \text{OD(sample)}}{\text{OD(blank control)}} \times 100\%$$

Hydroxyl radical scavenging activity test

The test was carried out according to the hydroxyl radical activity kit (Nanjing Jiancheng Biological Co., Ltd.), Vc was employed as a positive control, equal amount of deionized water was added into the blank control tube, and the absorbance value of each reaction solution at 550 nm was calculated by UV spectrophotometer.[24]

Antioxidant activity *in vivo*

Specific-pathogen-free Kunming mice (male: female = 50:50, provided by Sichuan Academy of Medical Sciences) weighing 18–22 g were used in this study. All experimental processes of animals were permitted by the Ethics Committee for Animal Experimentation of Sichuan Agricultural University and carried out in accordance with the Regulations of Experimental Animal Administration distributed by the State Committee of Science and Technology of the People's Republic of China.

The mice were fed at a temperature of 21°C ± 1°C, a relative humidity of 50%–60%, 12 h of light and dark cycles, and free contact to food and water. Forty mice were arbitrarily separated into four groups, comprising control group (normal saline group), high concentration polysaccharide group (3200 μg/mL), medium concentration polysaccharide group (1600 μg/mL), and low concentration polysaccharide group (800 μg/mL). Each group was induced by a single intraperitoneal injection of D-galactose (100 mg/kg/day body weight, D-galactose dissolved in 0.9% saline solution). D-galactose model control group was intraperitoneally injected with 0.2 mL normal saline (0.9% w/V). After 28 days, the mice were destroyed by cervical dislocation. Blood samples were composed of the eye socket of mice, centrifuged, and treated with serum. The activities of GSH-Px, SOD, and the content of MDA were measured.

Statistical analysis

The test results are articulated by mean ± standard deviation. Statistical analysis was conducted with SPSS 23.0 (SPSS Inc., Chicago, USA), and significant difference, very noteworthy difference, and enormously important difference were uttered by $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

RESULTS

Content of uronic acid and sulfate sugar in polysaccharides

DAP-A40, DAP-A60, and DAP-A80 were gained by alkaline water extraction and ethanol precipitation. The content of uronic acid in each polysaccharide sample is revealed in Table 1. It can be seen from Table 1 that the content of uronic acid in DAP-A60 is the uppermost and that in DAP-A80 is the bottommost. DAP-A40 had the highest content of sugar sulfate and DAP-A80 had the lowest content.

Molecular weight analysis

The three polysaccharides were all white-like powder. High-performance gel permeation chromatography presented a single peak. According to the standard curve of M_W, the M_W was intended by using Empower software [Table 2]. It can be seen from the table that the weight average M_W of the three polysaccharide samples is DAP-A40 > DAP-A60 > DAP-A80, in which DAP-A40 is an order of magnitude larger than DAP-A60 and DAP-A80, but there is no order of magnitude difference between DAP-A60 and DAP-A80. The number average M_W of the three polysaccharides was DAP-A60> DAP-A40> DAP-A80. The polydispersity index of the three samples was DAP-A40> DAP-A60> DAP-A80, which disclosed that

the distribution breadth of DAP-A60 and DAP-A80 was smaller than that of DAP-A40.

Analysis of polysaccharide fragments by infrared spectroscopy

According to the infrared spectra of the polysaccharide samples, there were clear absorption peaks at 3400/cm, 2900/cm, 1600/cm, and 1400/cm. The absorption peak near 3400/cm is the distinguishing peak of –OH stretching vibration. The absorption peak near 2900/cm is the characteristic peak of –CH stretching vibration. The absorption peaks near 1600/cm and 1400/cm were stretching vibration of carboxylate anion groups, which designated that carboxyl groups were existing in the samples. The infrared spectra also showed that the three polysaccharides had multiple absorption peaks at 1200/cm-1000/cm, which were characteristic peaks of C–OH side group and stretching vibration of C–O–C glycoside band. According to the above grades, all the three samples were polysaccharides.[25]

The infrared spectrum of DAP-A40 [Figure 1] expressed that the absorption peak near 1634/cm of DAP-A40 may be the characteristic peak of N–H angular vibration in primary amino group, secondary amino group, or amide group and stretching vibration of C = O in carbonyl group. The absorption peak here also specifies that there may be crystal water in DAP-A40. The peak near 1383/cm is the characteristic peak of symmetry stretching vibration of C = O in carbonyl group. The absorption peak near 1240/cm is the superposition of the characteristic peak of O-H variable angle vibration in –COOH and the characteristic peak of S = O stretching vibration in sulfuric acid group. At 1154/cm, 1080/cm, and 1023/cm, three characteristic peaks of pyranoside looked incessantly, representing that the monosaccharide group of DAP-A40 was pyranose. At 859/cm, the characteristic peak of α-glycosidic bond appeared.[26]

Table 1: Contents of uronic acid and sulfate sugar in polysaccharide of *Dendrobium aurantiacum* var. denneanum (Kerr)

Polysaccharide fragments (mg/g)	DAP-A40	DAP-A60	DAP-A80
Uronic acid content	168.40	292.20	34.87
Sugar sulfate content	198.62	139.13	76.77

DAP: Polysaccharide from *Dendrobium aurantiacum* var. denneanum (Kerr)

Table 2: Relative molecular weight of polysaccharides from *Dendrobium aurantiacum* var. denneanum (Kerr)

Polysaccharide fragments	Mw (Da)	Mn (Da)	Polydispersity (ρ)
DAP-A40	1.70×10^{5}	9.12×10^{3}	18.67
DAP-A60	5.78×10^{4}	1.10×10^{4}	5.25
DAP-A80	1.36×10^{4}	4.06×10^{3}	3.34

Mw: Molecular weight; DAP: Polysaccharide from *Dendrobium aurantiacum* var. denneanum (Kerr), Mw:Molecular weight, Da: Dalton, Mn: number-average molecular weight

The infrared spectrum of DAP-A60 [Figure 2] exhibited that the absorption peak near 1644/cm of DAP-A60 may be the characteristic peak of N–H angular vibration in primary amino group, secondary amino group, or amide group and stretching vibration of C = O in carbonyl group. The absorption peak here also directs that there may be crystal water in DAP-A60. At 1521/cm, the characteristic absorption peak of N–H variable angle vibration in the first-order amino group appears and the characteristic peak of C–O stretching vibration in –COOH seems at 1422/cm. The absorption peaks near 1243/cm are S = O stretching vibration in sulfuric acid group; three consecutive absorption peaks of pyranoside appear at 1157/cm, 1079/cm, and 1018/cm, and there are characteristic peaks of α-glycoside bond at 858/cm.

The infrared spectrum of DAP-A80 [Figure 3] illustrated that the absorption peak near 1643/cm of DAP-A80 may be the characteristic peak of N–H angular vibration in primary amino group, secondary amino group, or amide group and stretching vibration of C = O in carbonyl group. The absorption peak here also specifies that DAP-A80 has the characteristic peak of crystal water. The characteristic peak of C–O stretching vibration in –COOH looks at 1411/cm. The weak absorption peak near 1245/cm is S = O extending vibration in sulfuric acid group. The characteristic absorption peak of pyranoside appears at 1150/cm, 1077/cm, and 1039/cm.[27] The peak of 901/cm is the characteristic of glycosidic bond with β-configuration performed.

Antioxidant activity of polysaccharide fragments

Reducibility of polysaccharide fragments

The results of reducibility investigation are revealed in Figure 4. It can be seen from the figure that the positive control and the three polysaccharides have a definite decreased ability to ferric ions. However, the reducing ability of polysaccharides to Fe (el is different at different concentrations. When the concentration was in the range of 25–800 μg/mL, the reducing ability of DAP-A60 and DAP-A80 augmented with the increase of concentration, but it was visibly weaker than Vc. When

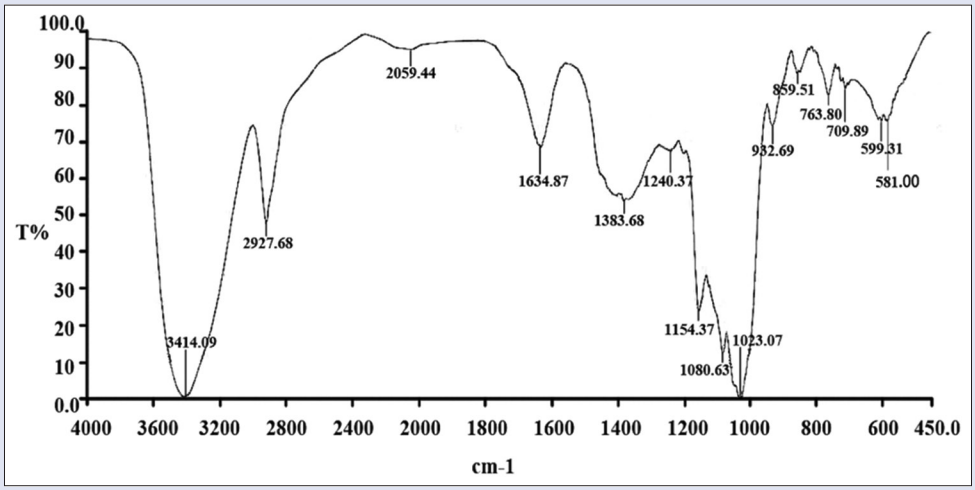

Figure 1: Infrared spectrum analysis of polysaccharide from DAP-A40

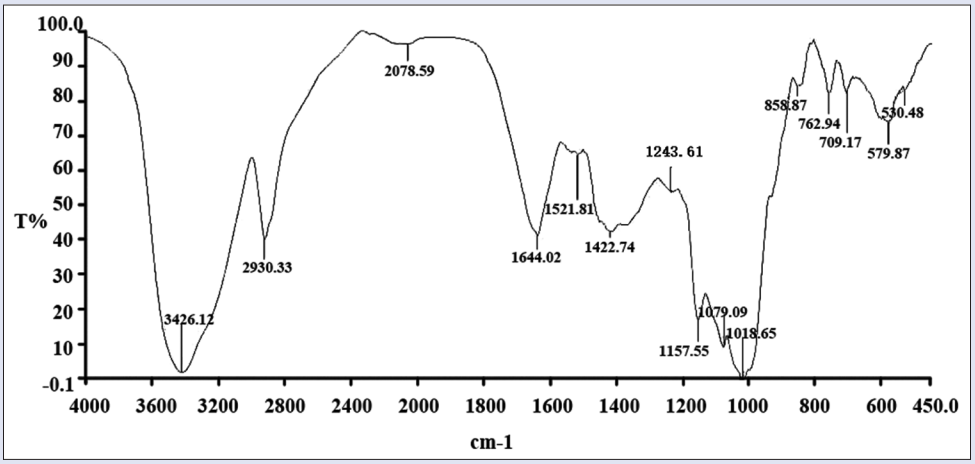

Figure 2: Infrared spectrum analysis of polysaccharide from DAP-A60

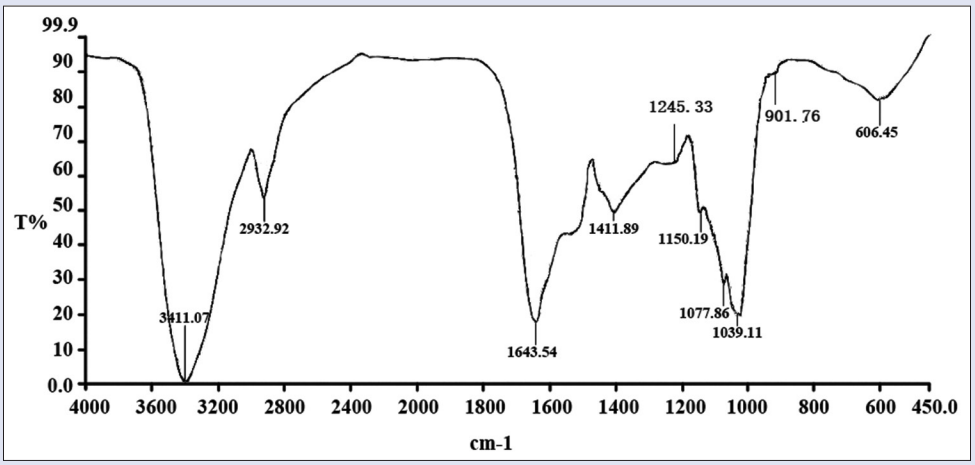

Figure 3: Infrared spectrum analysis of polysaccharide from DAP-A80

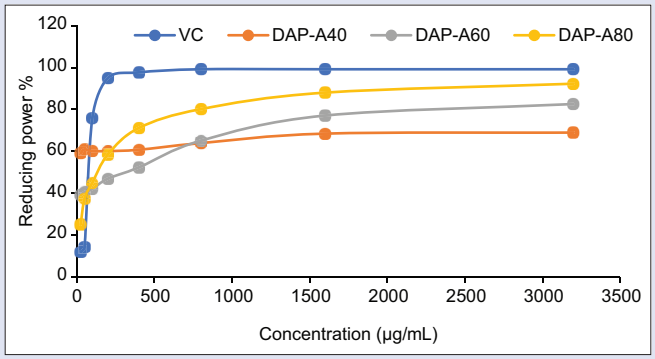

Figure 4: Reducing abilities of polysaccharide components

the concentration was higher than 800 μg/mL, the reducing ability of DAP-A60 and DAP-A80 increased slowly. When the concentration was 3200 μg/mL, the reducing ability of DAP-A80 was close to that of Vc ($P > 0.05$) and its half-maximal inhibitory concentration (IC_{50}) was 163.5 μg/mL [Table 3]. However, the reducing ability of DAP-A40 was lower than that of Vc and had no clear change with the surge of concentration.

It can be seen that the reducing ability of each polysaccharide sample is dissimilar. Among them, DAP-A80 has the solidest scavenging ability and its reduction ability is close to that of positive control at high concentration (3200 μg/mL). DAP-A40 had the feeblest reduction ability, which was much lower than that of the positive control.

Ability of polysaccharides to scavenge 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) free radicals

It can be seen from Figure 5 that the polysaccharide samples and Vc have confident scavenging ability to ABTS free radicals. When the concentration was in the range of 25–800 μg/mL, the scavenging ability of DAP-A60 and DAP-A80 to ABTS radical augmented with the increase of concentration, viewing a certain concentration dependence. When the concentration of DAP-A80 was 800 μg/mL, the scavenging capacity of DAP-A80 was close to that of Vc ($P > 0.05$) and its IC_{50} was 613.2 μg/mL [Table 3].

When the concentration of DAP-A80 was higher than 800 μg/mL, the scavenging ability of DAP-A80 to ABTS radical was near to that of Vc and almost no change was detected. However, the scavenging ability of DAP-A60 to ABTS radical augmented with the increase of concentration.

When the concentration of DAP-A60 was 3200 μg/mL, the scavenging capacity of DAP-A60 to ABTS free radical was 87.6%, which had no momentous difference compared with Vc ($P > 0.05$), and IC_{50} was 1839.8 μg/mL [Table 3]. However, DAP-A80 had the same scavenging capacity at the concentration of 800 μg/mL. Therefore, DAP-A80 has a stronger ability to scavenge ABTS radicals than DAP-A60. However, DAP-A40 had the weakest ability to scavenge ABTS free radicals. When the concentration was 3200 μg/mL, the scavenging rate of DAP-A40 was only 9.66%. The scavenging ability of Vc and the other two polysaccharide samples was much lower than that of DAP-A40 group. It can be seen that the scavenging ability of the polysaccharides to ABTS radical is diverse. Among them, DAP-A80 has the strongest scavenging ability and it has stretched the clearance ability of positive control at a low concentration (800 μg/mL). DAP-A40 had the weakest ability to eliminate ABTS.

Ability of polysaccharides to scavenge DPPH free radicals

The DPPH radical scavenging outcomes of polysaccharide samples and Vc are publicized in Figure 6. It can be seen from the figure that when the polysaccharide concentration is in the range of 25–800 μg/mL, the scavenging ability of each polysaccharide sample upsurges with the increase of the concentration, viewing a certain degree of concentration dependence. The positive control Vc showed a very strong scavenging capacity, even at a low concentration (25 μg/mL), its scavenging capacity could extent to 66.2%. When the concentration increased to 1600 μg/mL, scavenging ability on DPPH radical was no significant difference between DAP-A80 and Vc ($P > 0.05$) and the IC_{50} of DAP-A80 was 583.2 mg/mL [Table 3]. When the concentration was 1600 μg/mL, the scavenging ability of DAP-A60 and DAP-A40 on DPPH radical was also amplified to some extent, but it was much lower than that of Vc ($P < 0.05$). When the concentration was higher than 1600 μg/mL, the DPPH radical scavenging ability of DAP-A80 was slightly higher than that of Vc. The DPPH radical scavenging capacity of DAP-A60 was increased greatly, but it was still suggestively lower than that of Vc. The DPPH radical scavenging ability of DAP-A40 was still significantly lower than that of Vc ($P < 0.05$). It can be seen that the DPPH radical scavenging ability of each polysaccharide sample is different. The scavenging ability of DAP-A80 was the sturdiest. DAP-A40 had the weakest scavenging ability to DPPH free radicals, even at a high concentration (3200 μg/mL), its scavenging rate was only 22.49%.

Ability of polysaccharides to scavenge OH free radicals

Figure 7 indicates the hydroxyl radical scavenging results of three polysaccharide samples and Vc. It can be seen from the graph results that when the concentration of polysaccharides is in the range of 25–800 μg/mL, the scavenging ability of polysaccharides to hydroxyl radical increases with the increase of concentration, showing a certain concentration dependence. Among them, DAP-A80 enlarged the most. When the concentration was 800 μg/mL, the clearance rate of DAP-A80 reached the maximum value, close to Vc. The IC_{50} of DAP-A80 was 365.1 μg/mL [Table 3], which was much stronger than the other two polysaccharide samples (IC_{50} of 4538.0 and 4500.6 μg/mL, respectively). When the concentration sustained to increase, the clearance rate of DAP-A80 was near Vc and did not alteration. When the concentration continued to increase, the scavenging rate of DAP-A60 and DAP-A40 to OH radical augmented slowly. Although it also showed a concentration-dependent manner, its scavenging capacity was weak, even at high concentrations, it was significantly lower than that of positive control and DAP-A80 ($P < 0.05$). It can be seen that the scavenging ability of these three polysaccharide

Table 3: The half-maximal inhibitory concentration of different samples (μg/mL)

Sample	Reducing power	ABTS	DPPH	OH
DAP-A40	-	8080.8	7517.1	4500.6
DAP-A60	383.5	1565.1	1839.8	4538.3
DAP-A80	163.5	583.2	613.2	365.1

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic); DPPH: 1,1-diphenyl-2-picrylhydrazyl, DAP: Polysaccharide from *Dendrobium aurantiacum* var. denneanum (Kerr)

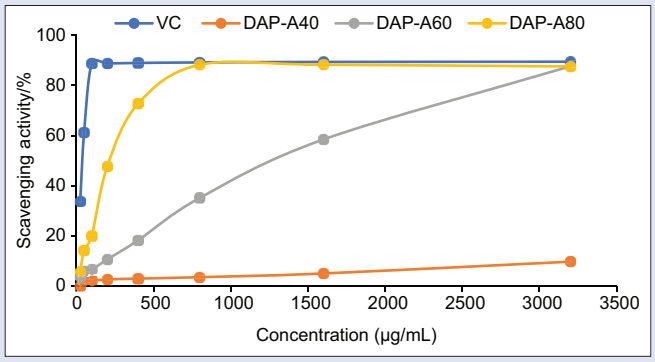

Figure 5: The abilities of polysaccharides to scavenge 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) free radicals

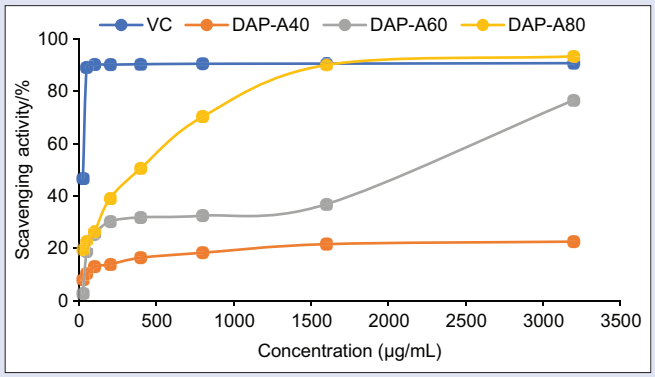

Figure 6: The abilities of polysaccharides to scavenge DPPH free radicals

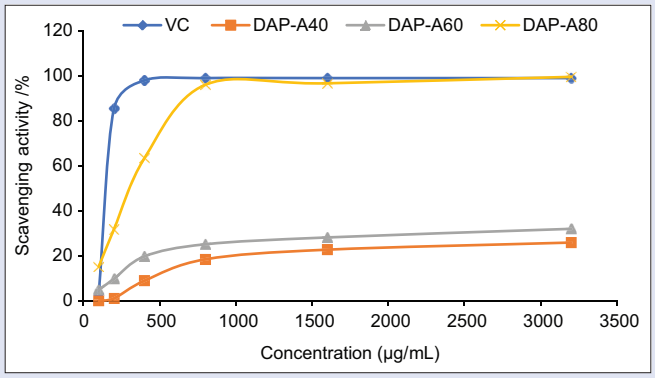

Figure 7: The abilities of polysaccharides to scavenge OH free radicals

fragments to OH radical is dissimilar. Among them, DAP-A80 has the strongest scavenging ability and it has grasped the clearance ability of positive control at a low concentration (800 μg/mL). However, DAP-A40 and DAP-A60 showed a weak scavenging effect on OH radicals.

Correlation analysis of antioxidant activity of polysaccharides

The relationship between the content of sulfated sugar and uronic acid, M_W, and antioxidant activity of three polysaccharide samples of *D. aurantiacum* var. denneanum (Kerr) is shown in Table 4. The M_W of polysaccharide was negatively connected with its capability to scavenge ABTS, DPPH, and OH free radicals and was significantly interrelated with the activity of scavenging ABTS and DPPH free radicals. The correlation coefficients were −0.988 and −0.994, respectively. However, the content of sulfate group was definitely correlated with ABTS, DPPH, and OH radical scavenging activities and the correlation coefficients were 0.915, 0.932, and 0.869, respectively. On the contrary, there was no relationship between the content of glucuronic acid and antioxidant capacity. At the same time, the reducing ability of polysaccharides has no correlation with M_W, sulfate sugar, and uronic acid. Therefore, the results displayed that the M_W and the content of sulfated sugar could ominously affect the free radical scavenging ability of polysaccharides.

Antioxidant activities *in vivo*

The marks of antioxidant experiment *in vitro* presented that DAP-A80 had the strongest antioxidant activity, so DAP-A80 was picked to estimate the antioxidant activity *in vivo*. The results are shown in Table 5. It can be seen from the table that there was no substantial change between DAP-A80 and the control at low concentrations. With the increase in the concentration, the effect of DAP-A80 on GSH-Px augmented progressively. The maximum activity was recorded at DAP-A80/H. DAP-A80 might enticingly recover the activity of GSH-Px ($P < 0.01$). Compared with the control group, DAP-A80 expressively augmented the activity of SOD at low concentration ($P < 0.05$). With the increase in polysaccharide levels, the effect of DAP-A80 on the activity of SOD is superior. According to the results, low levels of DAP-A80 might knowingly eradicate MDA from mouse serum compared with control and the higher the concentration of DAP-A80 was the more perceptible the effect of clear MDA.

Table 4: Relationship between the content of molecular weight, sulfate content, uronic acid, and the antioxidant activity

Antioxidant assays	Correlations		
	Molecular weight	Sulfate content	Uronic acid content
ABTS	−0.988	0.915	0.142
DPPH	−0.994	0.932	0.188
OH	−0.713	0.869	0.880
Reducing power	-	-	-

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic); DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 5: Antioxidant activities of the DAP-A80 *in vivo*

Groups	GSH-PX (U/mL)	SOD (U/mL)	MDA (nmol/mL)
Control	51.08±1.33	93.66±3.19	28.63±1.17
DAP-A80/H	100.26±2.67**	149.38±6.96**	7.16±0.48**
DAP-A80/M	71.59±1.49*	103.79±5.28	8.53±0.19**
DAP-A80/L	65.82±2.55	101.52±2.98	17.65±1.38*

*Versus control group, $P<0.05$; **Versus control group, $P<0.05$. Control (saline), DAP-A80/H (3200 μg/mL), DAP-A80/M (1600 μg/mL), and DAP-A80/L (800 μg/mL). SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; MDA: Malondialdehyde; DAP: Polysaccharide from *Dendrobium aurantiacum* var. denneanum (Kerr)

DISCUSSION

The monosaccharide groups of polysaccharides cover different numbers of active hydroxyl groups, which deliver active hydrogen or electrons. They can act as donors of hydrogen protons or electrons, straight quench free radicals, or inhibit the chain reaction of free radicals, so as to evade the damage of free radicals to cells. Their derivatives increase the active groups such as amino group and carboxyl group, which will advance the ability of scavenging free radicals.[15] In this study, three polysaccharide fragments showed different antioxidant activities *in vitro*. Among them, DAP-A80 had the stoutest antioxidant activity, especially at the high concentration (3200 μg/mL), its antioxidant capacity was near to the positive control Vc, and there was no significant difference ($P < 0.05$).

Combined with some reports, we reflect that DAP-A80 may quench free radicals or inhibit the chain reaction of free radicals, thus comprehending chemical antioxidant. Similar results are also imitated in other polysaccharides, such as the polysaccharides extracted from pistachio external hull have a palpable scavenging effect on ABTS free radical and DPPH free radical,[28] and the *Sagittaria trifolia* polysaccharide can knowingly scavenge hydroxyl, DPPH, and ABTS free radicals.[29]

Research displays that the biological activity of polysaccharides is carefully connected to their structural characteristics. Therefore, the relationship between the structural characteristics of polysaccharides and their capacity to scavenge free radicals was considered in this study. The results showed that the M_W of polysaccharide was negatively correlated with its ability to scavenge ABTS+, DPPH, and ·OH radicals and was ominously correlated with the activity of scavenging ABTS + and DPPH free radicals. The correlation coefficients were −0.988 and −0.994, respectively. This may be due to the higher the M_W, the worse the water solubility and the weaker the binding ability with the receptor, causing the poor adhesion of polysaccharides on target organs, which greatly lessens its effect.[8]

It is stated that the sulfated groups in polysaccharides typically affect their biological activities.[30] The results of this experiment also showed that the content of sulfate group in polysaccharide samples was positively correlated with ABTS, DPPH, and OH radical scavenging activities and the correlation coefficients were 0.915, 0.932, and 0.869, respectively. Therefore, the results indicated that the M_W and the content of sulfated sugar could pointedly affect the free radical scavenging ability of polysaccharides.

SOD and GPx are the foremost antioxidant enzymes in cells, which can aid cells to resist oxidation. In this study, DAP-A80 can surge cell viability, enhance the activities of antioxidant enzymes SOD and GPx, and diminish the content of MDA in cells with oxidative damage in a concentration-dependent manner. It has been stated that polysaccharides can not only scavenge free radicals as hydrogen proton or electron donors, but also bind to cell receptors as signal substances, activate antioxidant related signal pathways and regulate antioxidant enzymes, so as to play an antioxidant role. Hence, combined with the results of this experiment, we deduce that DAP-A80 can diminish the MDA and increase the activities of GSH-PX and SOD in mice. Similar results are also reproduced in other polysaccharides, such as *Hericium erinaceus* crude polysaccharide can reduce the activity of myeloperoxidase in gastric tissue, increase the activities of SOD and GPx, expand the antioxidant status of gastric tissue, and help to maintain the integrity of gastric mucosa.[31]

CONCLUSION

In this study, DAP-A40, DAP-A60, and DAP-A80 were acquired by alkaline aqueous solution extraction and ethanol precipitation. The content of glucuronic acid was tried. GPC analysis showed that the M_W of DAP-A40, DAP-A60, and DAP-A80 was 1.70×10^5 Da, 5.78×10^4 Da, and 1.36×10^4 Da, respectively. Infrared spectrum unveiled that DAP-A40 and DAP-A60 were α-configuration polysaccharides. The results exposed that DAP-A80 has a strong antioxidant activity *in vitro* and *in vivo*.

Ethics statements

All experimental processes of animals were approved by the Ethics Committee for Animal Experimentation of Sichuan Agricultural University and carried out in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

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Conflicts of interest

There are no conflicts of interest.

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