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Extraction, structural analysis and antioxidant activity of aloe polysaccharide

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ABSTRACT

Using fresh aloe as a raw material, aloe polysaccharide was prepared by the water extraction. After protein removal by sevage method, the aloe polysaccharide was refined by dialysis. The aloe polysaccharide was separated by diethylaminoethyl-52 (DEAE-52) cellulose column and Sephadex G-100 to obtain a single component of aloe polysaccharide. The molecular weight and monosaccharide components were determined by size exclusion chromatography-multi-angle laser light scatterer-differential refractive index detector (SEC-MALLS-RI) and high performance liquid chromatography (HPLC), respectively, and the structure was confirmed by nuclear magnetic resonance (NMR) spectrum. The molecular weight of aloe polysaccharide was 1.32×10^6 Da. The monosaccharide components were fucose (Fuc), arabinose (Ara), rhamnose (Rha), galactose (Gal), glucose (Glc), xylose (Xyl), mannose (Man), galacturonic acid (Gal-UA) and glucuronic acid (Gul-UA), and the proportions of each component were 0.37%, 7.52%, 3.46%, 10.23%, 26.99%, 1.61%, 35.02%, 14.25% and 0.55%, respectively. In order to obtain the structure-activity relationship (SAR) of aloe polysaccharide, the derivatives, including acetylated aloe polysaccharides (AAP), phosphorylated aloe polysaccharides (PAP) and carboxylated aloe polysaccharides (CAP) were synthesized. Results of antioxidant test *in vitro* showed that aloe polysaccharide and its derivatives had a good activity.

1. Introduction

Aloe vera is a perennial evergreen succulent herb of *Liliaceae*, originating in the tropical and subtropical regions of Africa [1–3]. In folk, *aloe vera* has been used for detoxification, constipation treatment and other diseases for thousands of years [4]. Results of modern pharmacological studies showed that the active ingredients in *aloe vera* have more than ten pharmacological effects, such as anti-bacterial, anti-inflammatory, anti-cancer, promoting wound healing, enhancing body immunity and so on [5–7]. *Aloe vera* is rich in a variety of biological activity substances and has been widely used in food, beauty, health care, medicine and other fields [8–10]. *Aloe vera* has complex chemical components, including anthraquinone, polysaccharides, amino acids, organic acids, vitamins, minerals and trace elements [11]. Polysaccharide was the main biological activity substance in *Aloe vera* [12–15]. The activity research results showed that aloe polysaccharide has many functions such as antioxidation, antitumor, antiradiation, antiaging, antibacteria and antiinflammatory, acquired immune deficiency syndrome (AIDS) prevention, immune regulation and immunity enhancement. At present, the extraction methods of aloe polysaccharide have been reported, such as hot water extraction, acid-base extraction, enzyme extraction and so on [16–21]. The structure and activity of aloe polysaccharide extracted by different methods were different. However, there are few reports on the molecular weight and monosaccharide components of aloe polysaccharides, but the molecular weight of polysaccharides has a certain effect on its activity. In addition the synthesis and activity of aloe polysaccharide derivatives have not been reported. In this research project, aloe polysaccharide was prepared from fresh aloe vera leaves by cold storage and water extraction, and the extraction process was optimized. Purified aloe polysaccharide was prepared through protein removal, dialysis, DEAE-52 and Sephadex G-100. After purified, aloe polysaccharide were derivatization, including acetylation, carboxymethylation and phosphorylation, were carried out. The antioxidant activities of aloe polysaccharides and their derivatives were studied, which provided some theoretical guidance for the study of their structure-activity relationship (SAR) and further research as drugs or functional food.

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2. Experiment part

2.1. Materials and method

Fresh aloe leaves were purchased from Shandong (Shangdong, China), in July 2021. The commercially available aloe vera leaves were identified by Professor Chen Guangying of Hainan Normal University and stored in the laboratory of Chongqing Normal University. All reagents were purchased from Aladdin (Shanghai, China).

2.2. Preparation of aloe polysaccharide (AP)

Aloe leaves → pretreatment → freezing treatment → soak → extracted filtration → centrifuged → concentrated → removed protein and nucleic acid → concentrated → dialysis → alcohol-precipitated → centrifuged → refined aloe polysaccharide

After removing the necrotic parts, the fresh aloe leaves were cleaned and naturally dried. Aloe leaves of 100 g were thoroughly crushed and then frozen in a refrigerator at -10 to -5 °C for 10–12 h to completely break the tissues and cells. The frozen aloe juice was soaked in 500 g water at normal temperature for 30 min, and filtered at normal pressure to collect the filtrate. Then the filter residue was extracted twice by the same method. The filtrates were combined and centrifuged at 4000 r/min for 5 min to collect the supernatant, which was to be concentrated under vacuum pressure (-0.08 MPa to -0.1 MPa) in the temperature range of 50 to 55°C to obtain the crude aloe polysaccharide solution. When the crude aloe polysaccharid solution was evaporated to 200 mL, the concentrate was stopped. Protein and nucleic acid in the crude polysaccharide solution were removed by sevage method, namely 40 mL chloroform and 10 mL *n*-butanol were added into the solution, and magnetic stirring at room temperature for 30 min. After centrifugation at 4000 r/min for 5 min, the supernatant was transferred to a dialysis bag (MW > 7000 Da) and treated with distilled water to obtain a relatively pure aloe polysaccharide solution. After dialysis, the refined aloe polysaccharide solution was mixed with 800 mL absolute ethanol in a 1000 mL beaker, which was placed at static condition for about 24 h. The alcohol-precipitated mixture was centrifuged at 4000 r/min for 5 min to collect the precipitate, which was dried in an oven at 50–55 °C for 12 h to obtain the refined aloe polysaccharide (AP).

2.3. Purification of aloe polysaccharide

The refined aloe polysaccharide (5 mg/mL, 20 mL) was separated in a DEAE-52 cellulose column (2.5 × 25 cm) with distilled water and NaCl (0.05, 0.10, 0.50, 1.00 mol/L) solution at the rate of 2 mL/min. The eluent was collected every 5 mL a tube and the polysaccharides content was determined by phenol sulfuric acid method. According to the concentration, the eluate with high total sugar content was combined and then concentrated. The polysaccharide concentrate was further isolated by a Sephadex G-100 column (2.5 cm × 50 cm) with water and collected every 5 mL. Each tube eluent was monitored again by the phenol-sulfuric acid method, then the homogeneous components were combined and lyophilized to generate pure aloe polysaccharide.

2.4. Determination of molecular weight of aloe polysaccharide

The homogeneity and molecular weight of various fractions were measured using size exclusion chromatography-multi-angle laser light scatterer-differential refractive index detector (SEC-MALLS-RI). The weight- and number-average molecular weight (Mw and Mn) and polydispersity index (Mw/Mn) of various fractions in 0.1 mol/L $NaNO_3$ aqueous solution were measured on a DAWN HELEOS-II laser photometer equipped with three tandem columns that was held at 45 °C using a model column heater. The flow rate was 0.4 mL/min. A differential refractive index detector was simultaneously connected to give the concentration of fractions and the dn/dc value. The dn/dc value of the fractions in 0.1 mol/L $NaNO_3$ aqueous solution was determined to be 0.141 mL/g.

2.5. Determination of monosaccharide composition

The monosaccharide composition of aloe polysaccharide was analyzed by high performance liquid chromatography (HPLC). 20 mg of aloe polysaccharide was hydrolyzed with 5 mL 2 mol/L TFA at 120 °C for 2 h. Vacuum-dried monosaccharide sample hydrolyzate was redissolved in distilled water (5 mL) after TFA was removed with methanol. 400 μL of polysaccharide hydrolyzate, 400 μL of PMP methanol solution (0.5 mol/L) and NaOH (0.3 mol/L) were reacted at 70 °C for 2 h, followed with 400 μL of HCl (0.3 mol/L) for neutralization at room temperature. The standards monosaccharide mixed solution (400 μL, 0.4 mg/mL) was analyzed by HPLC as described method. HPLC was performed on an Agilent 1100 (Agilent Technologies Inc. USA), fitted with Waters Acquity BEH C_{18} (2.1 × 50 mm, 5 μm) at 35 °C with UV detection at 250 nm. The mobile phase consisted of 50 mM ammonium acetate and 100% acetonitrile at a flow rate of 0.4 mL/min.

2.6. Preparation of acetylated aloe polysaccharide (AAP)

The pure aloe polysaccharide of 1 g was dissolved with 20 mL distilled water in a 250 mL clean bottom flask by magnetic stirring. At room temperature, the 1.2 mL $(CH_3CO)_2O$ was added slowly under stirring, in the process of which, the pH value of the reaction solution was constantly adjusted with 10% NaOH to control it at 9-10. Then, the reaction mixture was stirred at room temperature for 1 h to complete acetylation. Next, the reaction mixture was placed in a MW > 7000 Da dialysis bag for dialysis treatment. After dialysis, the remaining dialysate was mixed with 100 mL of 95% ethanol in a 250 mL bottom flask and precipitated for 24 h. The mixture was centrifuged at 4000 r/min for 5 min to collect the precipitate, which was dried in an oven at 50°C–55°C for 12 h to acquire the purified acetylated aloe polysaccharide (AAP).

2.7. Preparation of phosphorylated aloe polysaccharide (PAP)

H_3PO_4 of 5 mL with 1 mL *n*-butanol was stirred fully in a 250 mL clean round bottom flask. Under stirring, the pure aloe polysaccharide of 1 g was added slowly to the solution in batches at room temperature. The flask was placed in a water bath at 50°C and the reaction was continued with stirring for 5 h. After dilution with 20 mL distilled water, the solution was dialyzed in a MW > 7000 Da dialysis bag. Polysaccharide reaction solution of the remaining dialysate was precipitated for 24 h in a 250 mL bottom flask by adding 100 mL 95% ethanol. The alcohol-precipitated mixture was centrifuged at 4000 r/min for 5 min to collect the precipitate, which was dried in a drying oven at 50–55°C for 12 h.

2.8. Preparation of carboxymethylated aloe polysaccharide (CAP)

Chloroacetic acid of 6 g was dissolved in 10 mL of isopropanol for standby. Then, 1 g pure aloe polysaccharide was dissolved with 20 mL 10% sodium hydroxide solution by continuously magnetic stirring in a 250 mL clean bottomed flask. By magnetic stirring, the prepared chloroacetic acid-isopropyl alcohol solution was slowly added to the round bottom flask at normal temperature. The flask

was placed in to a water bath and reacted at 60 °C with continue stirring for 5 h. After reaction, the liquid was cooled to room temperature and then diluted with 20 mL distilled water. The diluted reaction solution was dialyzed in a MW > 7000 dialysis bag. By adding 200 mL 95% ethanol, polysaccharide of the remaining dialysate was precipitated for 24 h in a 500 mL round-bottom flask. The alcohol-precipitated mixture was centrifuged at 4000 r/min for 5 min to collect the precipitate, which was dried in a drying oven at 50-55°C for 12 h to acquire the purified carboxymethylated aloe polysaccharide (CAP).

2.9. Analysis of sugar content

The phenol-sulfuric acid method was used to measure sugar content of AP, AAP, PAP and CAP.

2.10. Analysis of degree of substitution

2.10.1. Degree of substitution-acetyl

The acetyl group (CH_3CO-) substitution degree of acetylated aloe polysaccharides was determined by acid-base titration method.

$$DS = \frac{132A}{4300 - 42A} \tag{2-1}$$

2.10.2. Degree of substitution-phosphate

The phosphate substitution degree of phosphorylated aloe polysaccharide was determined by ammonium phosphomolybdate method.

$$DS = \frac{5.23P}{100 - 3.32P} \tag{2-2}$$

2.10.3. Degree of carboxymethyl substitution

The degree of carboxymethyl substitution of carboxymethylated polysaccharides was determined by acidity meter.

$$\mathrm{E}(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \tag{2-3}$$

2.11. NMR spectrum analysis

NMR spectra of the aloe polysaccharide and its derivatives samples were recorded with a JNM-ECZ600R (JEOL, Japan). samples of 25 mg were dissolved in D_2O, and the ^{13}C NMR, DEPT 135, COSY, HSQC, HMBC and ^{31}P NMR spectra were recorded at 25°C.

2.12. Determination of antioxidant activity

2.12.1. Determination of superoxide anion (O_2^-) clearance rate

The polysaccharide sample solution of different concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL) were prepared. And the solution of 2.0 mL with 0.5 mL 50 mmol/L potassium phosphate buffer (pH7.8) and 0.1 mL 10 mmol/L hydroxylamine hydrochloride solution was mixed well and kept at 25 °C for 20 min. Then, the mixture added with 1mL 58 mmol/L *p*-aminobenzene sulfonic acid solution and 1 mL 7 mmol/L α-naphthylamine solution reacted at 30 °C for 30 min. After reaction, equal volume trichloromethane was added to the solution to extracted the pigment and centrifuged at 10,000 r/min for 3min. The pink aqueous phase of the upper layer was used to determine the absorbance at 530 nm.

$$\mathrm{E}(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \tag{2-4}$$

2.12.2. Determination of hydroxyl radical (·OH) scavenging rate

Polysaccharide sample solution of different concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL) were prepared. In a colorimetric tube, the polysaccharide solution of 1.0 mL was mix with 5 mL 9 mmol/L $FeSO_4$ and 5 mL 9 mmol/L ethanol-salicylic acid successively. Then, an appropriate amount of deionized water and mL 8.8 mmol/L H_2O_2 were added to the mixture and stirred well, which was then heated in water bath at 37 °C for 15 min to measure the absorbance at 510 nm.

$$\mathrm{E}(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \tag{2-5}$$

2.12.3. Determination of anti-lipid peroxidation capacity

The polysaccharide sample solution of different concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL) were prepared, and 1.0 mL of which was took out, respectively to incubate at 37°C for 10 min. After adding with 0.1 mL 6 mmol/L $FeSO_4$, it reacted in a water bath at 37°C for 1 h. The reaction was stopped by adding 1 mL 15% trichloroacetic acid. Then, 1 mL 0.67% thiobarbituric acid (TBA) was added to the solution and boiled for 15 min. After cooling, the solution was centrifuged to acquire the supernatant, which was used to measure the absorbance at 532 nm.

$$\mathrm{E}(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \tag{2-6}$$

2.12.4. Determination of scavenging ability of DPPH free radical

Polysaccharide sample solution of different concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL) were prepared. In plug test tube, 4 mL DPPH ethanol solution (mass fraction of 0.004%) was quickly added to the sample, which was mixed well and reacted for 30 min at room temperature in dark environment. The absorbance of the solution was determined at 517 nm, and Vc with equal concentration was used as positive control. The test was repeated for 3 times in each group to calculate the average value.

$$\mathrm{E}(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \tag{2-7}$$

2.12.5. Determination of reducing capacity

The polysaccharide sample solution of different concentrations (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL) were prepared, and 1.0 mL of which was mixed with 2.5 mL phosphoric acid buffer solution and 2.5 mL 1% potassium ferricyanide solution. After mixing, it was placed in a 50°C water bath and reacted for 20 min. Then, 2.5 mL 10% trichloroacetic acid was added to the solution and kept at room temperature for 10 min. Finally, the above solution was mixed with 2.5 mL distilled water and 0.5 mL 0.1% ferric chloride solution and reacted for 10 min to determine the absorbance at 700 nm.

$$\mathrm{E}(\%) = \frac{1 - (A_1 - A_0)}{A_2} \times 100\% \tag{2-8}$$

2.13. Analysis of data

All data were expressed as mean ± standard deviation (SD). The experiment was repeated three times. ANOVA was used to analyze RSM results, and the difference was considered significant at $P < 0.05$.

3. Results and discussion

3.1. Extraction, total sugar content and degree of substitution

As a perennial evergreen herb, aloe was rich in a variety of biological activity substances that was beneficial to human body, and mainly used as food, beauty, medical and other important raw materials. Aloe polysaccharide was the main substance in aloe gel, and its extraction methods mainly included traditional extraction methods (including water extraction, acid extraction, alkali extraction and enzyme extraction) and auxiliary extraction methods (including ultrasonic assisted extraction, microwave assisted extraction and enzyme assisted extraction). With the development of science and technology, other new extraction methods are derived. In this study, fresh aloe was used as the material for extracting polysaccharides. First, aloe was fully mashed by a homogenizer, and then frozen in a refrigerator at -10°C to -5°C. Aloe polysaccharide was extracted by water immersion at normal temperature. Af-

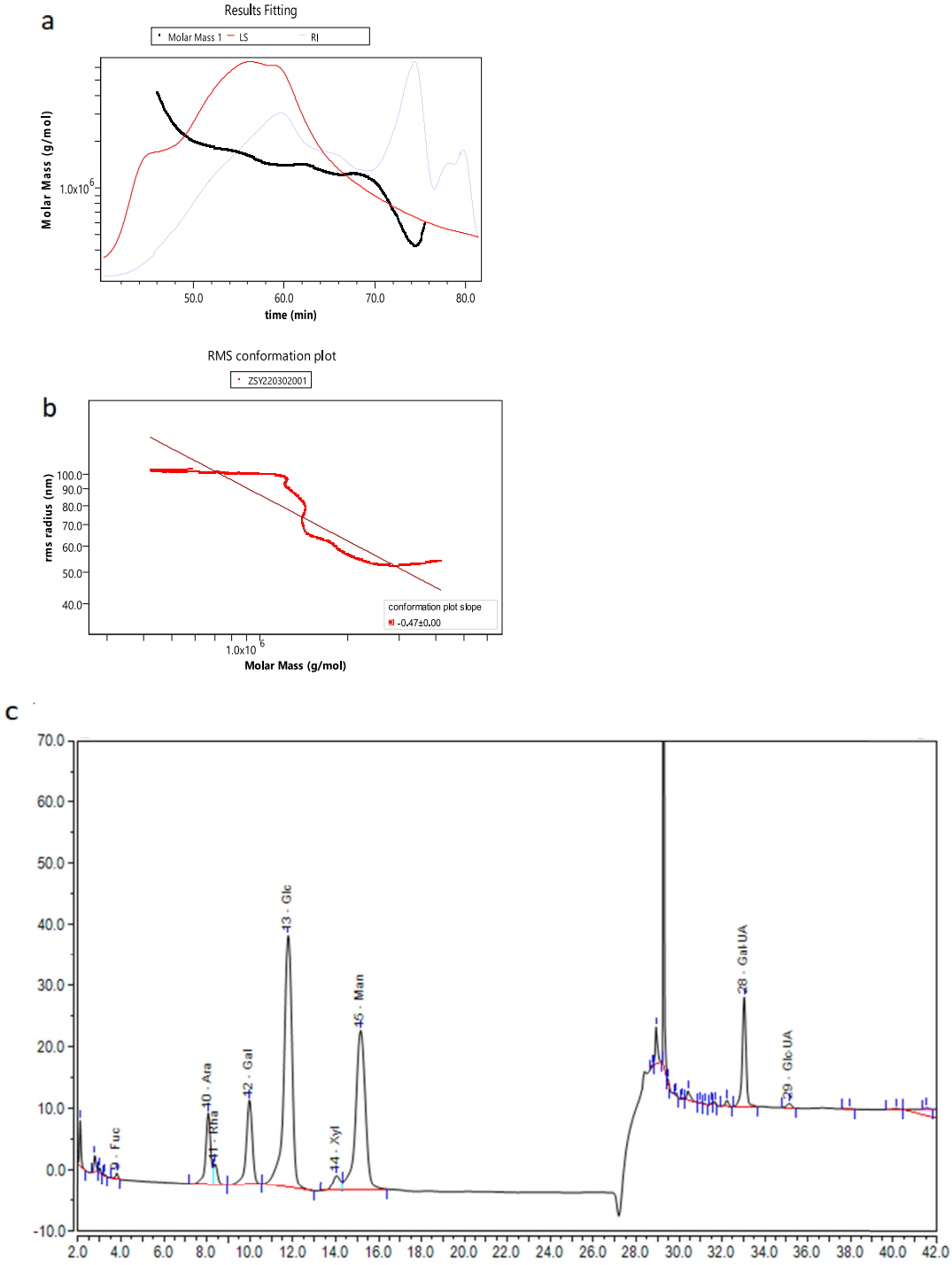

Fig. 1. The molecular weight determination and monosaccharide composition of aloe polysaccharide. (a) absolute molecular weight analysis of aloe polysaccharide, red line: multi-angle laser light scattering signal; blue line: differential signal; black line: molecular weight fitted by two signals; (b) molecular configuration analysis diagram of aloe polysaccharide; (c) chromatographic diagram of monosaccharide composition of aloe polysaccharide.

Table 1
Total sugar content, yield and degree of substitution of aloe polysaccharide and its derivatives ($n = 12$).

	Samples			
	AP	AAP	PAP	CAP
Yield (%)	0.51	84.56	38.34	81.61
Sugar content (%)	97.1	91.35	80.68	90.93
DS	—	0.96	0.39	0.91

ter protein removal, dialysis, alcohol precipitation, drying and other operations, the refined aloe polysaccharide was obtained. As can be seen from Table 1, the extraction rate of aloe polysaccharide was 0.51% (calculated by aloe wet weight). The aloe polysaccharides obtained by freezing treatment and then soaking in water at room temperature can maintain the structure of polysaccharides to the greatest extent and reduce the hydrolysis of polysaccharides caused by heating. In the process of derivatization, we prepared acylated aloe polysaccharides (AAP), phosphorylated aloe polysaccharides (PAP) and carboxymethylated aloe polysaccharides (CAP), with yields of 84.56%, 38.34% and 81.61%, respectively. The higher yields of AAP and CAP may be related to reaction conditions and reactants. Phenol-sulfuric acid method, 3, 5-dinitrosalicylic acid colorimetric method, and anthrone-sulfuric acid method were often used for the determination of total sugar content in plant polysaccharides. Among them, phenol-sulfuric acid method has a good stability and high accuracy, which was favored by most researchers and widely used in the determination of total sugar content in polysaccharides. In this study, total sugar content of the aloe polysaccharide and its derivatives (including AAP, PAP and CAP) was determined by phenol-sulfuric acid method. The determination results of total sugar content were shown in Table 1. It can be seen from the data that the total sugar content of aloe polysaccharide could be reach 97.3%, and the total sugar content of derived AAP, PAP and CAP were 91.35%, 80.68% and 90.93%, respectively. The total sugar content of aloe polysaccharide and its derivatives was ideal and the loss of total sugar was small. Degree of substitution (DS) refers to the number of active hydroxyl groups on each glucose unit (glucoside) in polysaccharides, and was an indicator to measure the difficulty of substitution. In order to study the substitution of aloe polysaccharide derivatives, the degree of

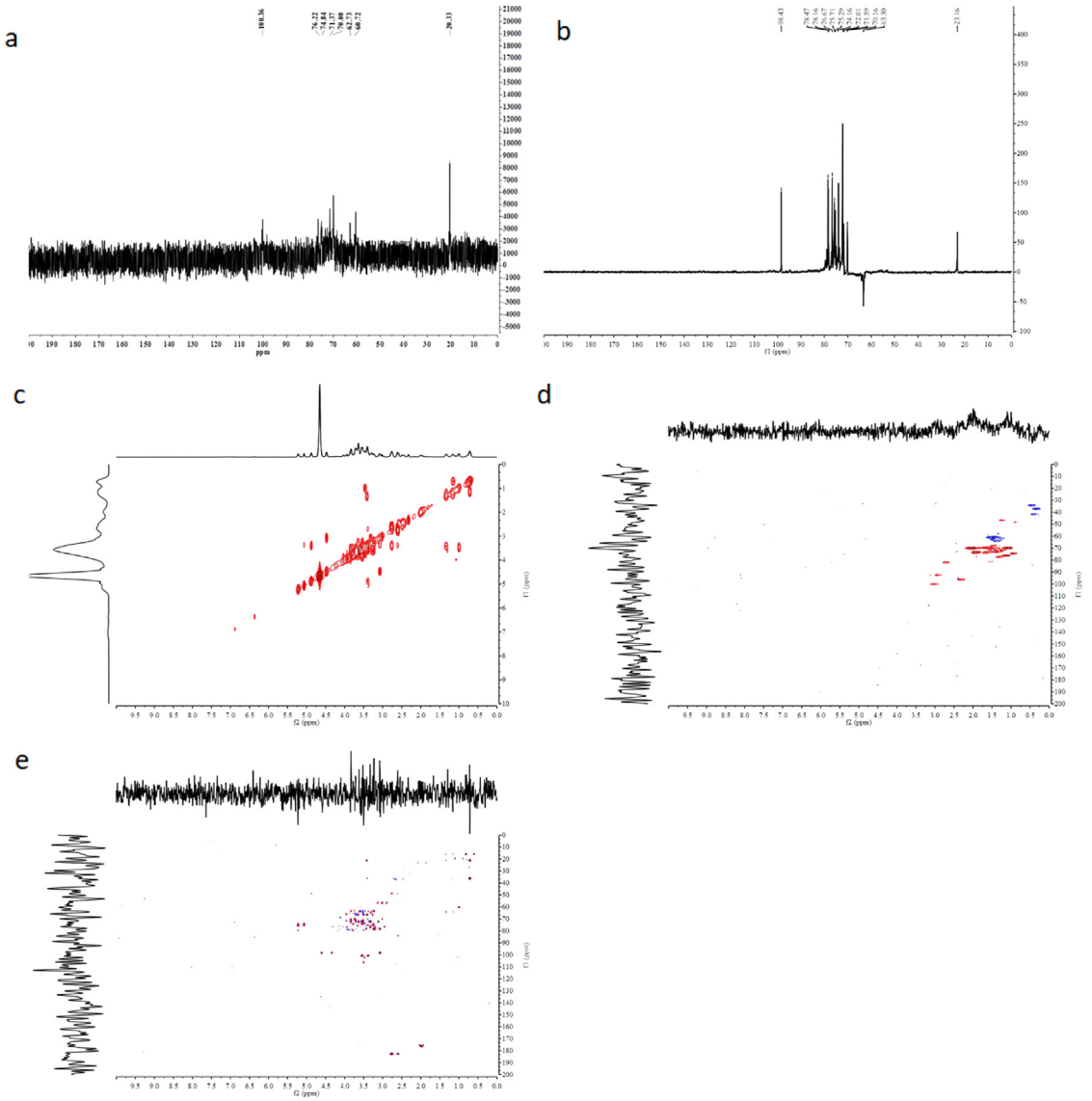

Fig. 2. The NMR spectrum analysis of aloe polysaccharide. (a) ^{13}C NMR spectrum of AP; (b) DEPT 135 spectrum of AP; (c) COSY pectrum of AP; (d) HSQC spectrum of AP; (e) HMBC spectrum of AP.

substitution of aloe polysaccharide derivatives was determined. As can be seen from Table 1, the degree of substitution of AAP, PAP and CAP were 0.96, 0.39 and 0.91, respectively. Among of them, the degree of substitution of AAP was the highest. In the derivatization process of aloe polysaccharides, reagents used, reaction time, reaction temperature and other factors affected the degree of substitution. Reagents used for acetylation and carboxymethylation were highly activity and the reaction conditions were mild, resulting in a higher degree of substitution. Phosphoric acid used in the phosphorylation process had weak activity, resulting in a low degree of substitution.

3.2. Molecular weight and monosaccharide composition determination of aloe polysaccharide

In order to determine the molecular weight and monosaccharide component of the purified aloe polysaccharide, we further separated and purified the polysaccharide. The purified aloe polysaccharides were separated by DEAE-52 cellulose column and Sephadex G-100 to obtain uniform aloe polysaccharide. GPC-RI-MALS method was an effective method for molecular weight determination of the polysaccharides. Unlike small molecular weight compounds, polysaccharides have no a fixed molecular weight, and are the mixtures of homologues with different molecular weights. The molecular weight of polysaccharides was in-deed an average and a concept of distribution. The average molecular weight of aloe polysaccharide detected by GPC-RI-MALS method was 1.32×10^6 Da (Fig. 1a-b). In Fig. 1a, the red, blue and black line were multi-angle laser light scattering signal, differential signal and molecular weight fitted by those two signals, respectively. The monosaccharide composition of aloe polysaccharide was measured by HPLC, and the results were showed in Fig. 1c. Aloe polysaccharide was mainly composed of Fuc, Ara, Rha, Gal, Glc, Xyl, Man, Gal-UA and Gul-UA, with the percentages of 0.37%, 7.52%, 3.46%, 10.23%, 26.99%, 1.61%, 35.02%, 14.25% and 0.55%, respectively. These data clearly demonstrated that aloe polysaccharide was a heterogeneous polysaccharide with Glc and Man as the dominant constituent.

3.3. The ^{13}C NMR DEPT 135, COSY, HSQC and HMBC analysis of aloe polysaccharide

^{13}C NMR spectrum was a commonly used means of characterization [22–32]. In order to confirm the successful derivatization of aloe polysaccharide and determine the structure of it and its derivatives, ^{13}C NMR spectrum was used to characterize. Fig. 2a shows the ^{13}C NMR spectrum of aloe polysaccharide. The chemical shift of C1 was at 100.36 ppm. There were β-L type or α-D-type glycosidic bonds in aloe polysaccharide. Peaks in the range of 60.72–76.22 ppm were the chemical shift of C2-C6. From the ^{13}C NMR spectrum of aloe polysaccharide, absorption peak at 20.33 ppm was the chemical shift of methyl carbon (-CH_3) during methylation, indicating that there was a -CH_3 on the sugar ring or sugar chain of aloe polysaccharide. DEPT spectrum was a kind of spectrograph used to distinguish the carbon of primary, secondary, tertiary and quaternary in NMR, and has certain application value in the structure resolution of polysaccharides. As can be seen from the spectra of DEPT of aloe polysaccharides (Fig. 2b), there were positive signal peaks at 98.43 ppm, 70.16–78.47 ppm and 23.16 ppm, corresponding to C1, C2-C5 and -CH_3 signal peaks. In the DEPT spectrum chart, 63.30 ppm was found to be the negative signal peak, which corresponded to the C6 signal peak. The results of DEPT of aloe polysaccharides were consistent with ^{13}C NMR. To obtain detailed structural information of aloe polysaccharides, 2D NMR spectrum including COSY, HSQC and HMBC were analyzed (Fig. 2c-e). 2D NMR spectrum structure analysis shows that H1/C1 (4.60/98.41 ppm), H2 (3.54 ppm) 3.54, H3 (3.23 ppm), H4/C4 (3.62/72.29 ppm), H5 (3.50 ppm), H6 (3.03 ppm) signal of

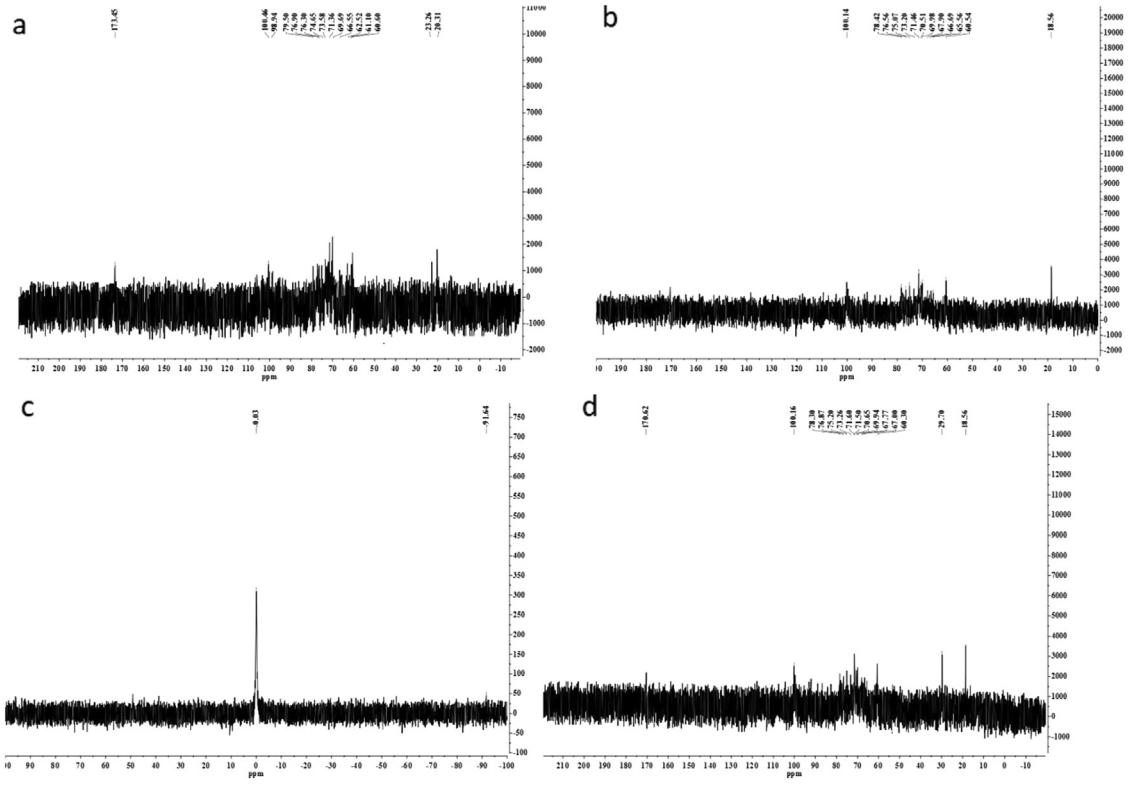

Fig. 3. The NMR spectra analysis of aloe polysaccharide derivatives. (a) ^{13}C NMR spectrum of AAP; (b) ^{13}C NMR spectrum of PAP; (c) ^{31}P NMR spectrum of PAP; (d) ^{13}C NMR spectrum of CAP.

carbon signs, it indicates the existence of →4-α-D-Glcp-(1→. In addition, there was a carbon signal at 21.06 ppm, and there was a linkage relationship with C6, indicating that the methyl group was connected with the sugar chain.

3.4. The ^{13}C NMR and ^{31}P NMR analysis of aloe polysaccharide derivatives

In order to obtain more aloe polysaccharide derivatives, we synthesized acetylated aloe polysaccharide (AAP), phosphorylated aloe polysaccharide (PAP) and carboxymethylated aloe polysaccharide (CAP) from aloe polysaccharide. Fig. 3a and 3b were the ^{13}C NMR spectrum of AAP and PAP, respectively, and Fig. 3d and 3c were ^{13}C NMR spectrum of CAP and ^{31}P NMR spectrum of PAP, respectively. In Fig. 3a, there was an absorption peak at 173.45 ppm, indicating that a carbonyl group (-CO-) existed here. The chemical shift of C2-C6 was 60.60–79.50 ppm, which had little change compared with aloe polysaccharide (C2-C6 was 60.72–76.22 ppm). Chemical shift of C1 was at 98.94–100.46 ppm, and 20.31 ppm and 23.26 ppm were the chemical shift signal of -CH_3. There were two methyl groups, and one was from the methylation of aloe polysaccharide itself and the other from the acetylation. In Fig. 3b, compared with aloe polysaccharide, the ^{13}C NMR spectrum after phosphorylation was basically unchanged. Among them, 60.54–78.42, 100.14 and 18.56 ppm were the chemical shift of C2-C6, C1 and methyl carbon in -CH_3, respectively. In Fig. 3c, there were absorption peaks at 0.03 ppm and -91.64 ppm, indicating that aloe polysaccharides had been phosphorylated. In Fig. 3d, there was an obvious chemical shift signal at 170.62 ppm, which was the characteristic signal peak of carbonyl group. The chemical shift of C2–C6 in the range of 60.30-78.30 ppm had little change compared with aloe polysaccharide. The peaks at 100.16, 18.56 and 29.70 ppm were chemical shift signal of C1, methyl and carboxymethylated methylene carbon, respectively.

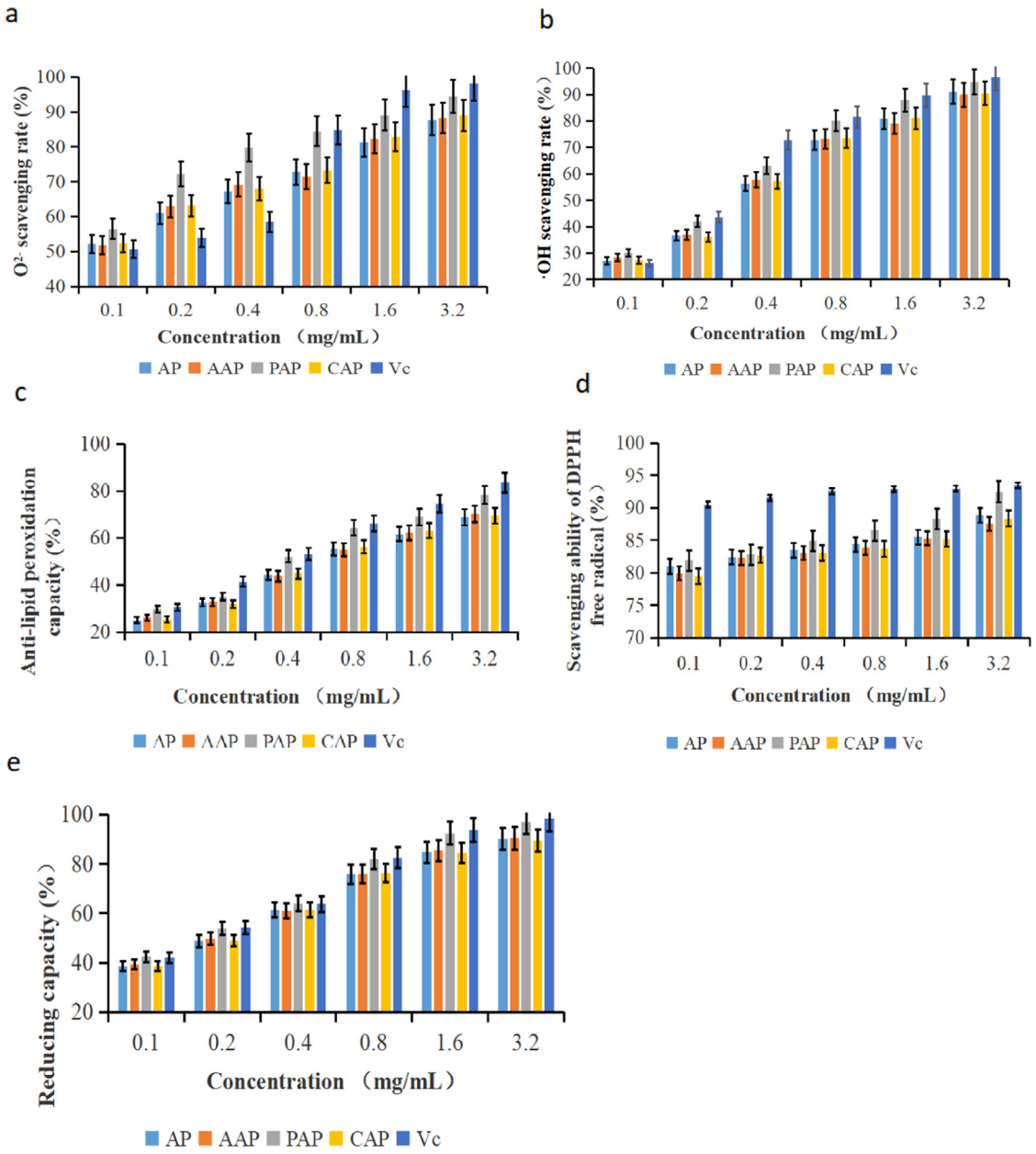

Fig. 4. The antioxidant activity *in vitro* of aloe polysaccharide and its derivatives. (a) superoxide anion scavenging rate; (b) hydroxyl radical scavenging rate; (c) anti-lipid peroxidation capacity; (d) DPPH scavenging capacity; (e) reducing capacity.

3.5. *The antioxidant activity* in vitro

Aloe was rich in various biological activity substances, among which aloe polysaccharide was the main biological activity component of the aloe gel. Aloe polysaccharides were a large group of macromolecular compounds with different physiological functions. The monosaccharide structure analysis showed that they were mainly composed of mannose, galactose, glucose, xylose, arabinose and rhamnose, and the contents of mannose, galactose and glucose were in the majority. Polysaccharide has immunological regulation, anti-tumor, liver protection, anti-radiation, anti-ulcer, anti-oxidation, anti-aging, hypoglycemic and other health care functions [33–43]. Due to the different methods of extraction, separation and purification of aloe polysaccharide, there are some differences in the content, dosage and pharmacological effect of aloe polysaccharide used in pharmacological experiments, which affect the evaluation of pharmacological effect of the aloe polysaccharide. With the further study on the biological activities of aloe polysaccharides, the mechanism and function factors will be more clearly, and its application field will be wider. In Fig. 4a, aloe polysaccharides and derivatives showed superior activity to Vc at low concentration ($p < 0.05$). In Fig. 4b, aloe polysaccharide and its derivatives showed similar activity to Vc in terms of hydroxyl free radical scavenging ability at the same concentration ($p < 0.05$). In Fig. 4c, aloe polysaccharide and its derivatives also showed a good activity in anti-lipid peroxidation capacity in the same activity as Vc at the same concentration ($p < 0.05$). In Fig. 4d, aloe polysaccharide and its derivatives also showed a good activity in terms of DPPH free radical scavenging ability, which was lower than that of the control Vc at low concentration, while equivalent to that of Vc at high concentration ($p < 0.05$). In Fig. 4e, the reducing ability of aloe polysaccharide and its derivatives were similar to that of Vc at the same concentration, however, the phosphorylated aloe polysaccharide was superior to Vc ($p < 0.05$). The activity of PAP was superior to that of aloe polysaccharide and AAP and CAP were basically at the same level as aloe polysaccharide.

4. Conclusion

In this study, refine aloe polysaccharide were prepared by homogenization, refrigeration, water extraction, concentration, alcohol precipitation, protein removal, dialysis, drying and other steps. The purified aloe polysaccharides were separated by DEAE-52 cellulose column and Sephadex G-100 to obtain a single component of aloe polysaccharide. Molecular weight and monosaccharide components of the purified aloe polysaccharide were determined by GPC-RI-MALS and HPLC, respectively. The results showed that the molecular weight of aloe polysaccharide was 1.32×10^6 Da, and the monosaccharides were mainly composed of Glc, Man and Gal-UA, with the percentages of 26.99%, 35.02% and 14.25%, respectively. The structure was characterized by ^{13}C NMR, DEPT 135, COSY, HSQC and HMBC spectrum, results of which showed that the aloe polysaccharide the possible structure was →4-α-D-Glc*p*-(1→. At the same time, we further synthesized aloe polysaccharide derivatives, including AAP, PAP and CAP. Results of antioxidant test *in vitro* showed that aloe polysaccharide and its derivatives had a good activity. This study provided theoretical guidance for the SAR study of aloe polysaccharide and its derivatives and their further research as drugs or functional food.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Shiyang Zhou: Writing – original draft. **Gangliang Huang:** Writing – review & editing.

Data availability

The authors do not have permission to share data.

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