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Preparation, structural characterization and in vitro activity of ginger polysaccharide

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Abstract

A polysaccharide was extracted from the fresh ginger by water extraction and alcohol precipitation. A homogeneous polysaccharide was obtained by Sevage deproteinization, dialysis, DEAE-52 ion exchange chromatography and Sephadex G-100 gel column chromatography. The molecular weight of the ginger polysaccharide was determined by high performance gel permeation chromatography. The monosaccharide composition, scanning electron microscope and other physicochemical parameters of the ginger polysaccharide were further determined. The spectral properties of the ginger polysaccharide were investigated by ultraviolet and infrared spectroscopy. On this basis, methylation analysis, 1D and 2D NMR analysis were used to investigate its possible chemical structure. The results showed that the ginger polysaccharide group was divided into neutral polysaccharide components with high homogeneity, $M_w = 35.52$ kDa, $M_n = 216.56$ kDa and the sugar content was 93.5%. The ginger polysaccharide is mainly composed of Fuc, Ara, Rha, Gal, Glc, Xyl, Man, Gal-UA and Glc-UA, the amount of substance ratio was 1.01:1.96:9.54:14.82:64.20:3.84:2.07:1.41:1.16. To study the structure–activity relationship of ginger polysaccharide, acetylated ginger polysaccharide and phosphate ginger polysaccharide were prepared by chemical methods in this study. The results of antioxidant activity in vitro showed that the ginger polysaccharide and its derivatives had good biological activity in freeing ABTS + radical scavenging, freeing hydroxyl radical scavenging, freeing DPPH radical scavenging and reducing ability. Interestingly, the results showed that the ginger polysaccharide had a certain inhibitory effect on the growth of *E. coli* in vitro. In general, this study provides a certain experimental basis and reference for the further research on the activity of ginger polysaccharide and its derivatives.

Keywords Ginger polysaccharide, Chemical structure, Biological activity

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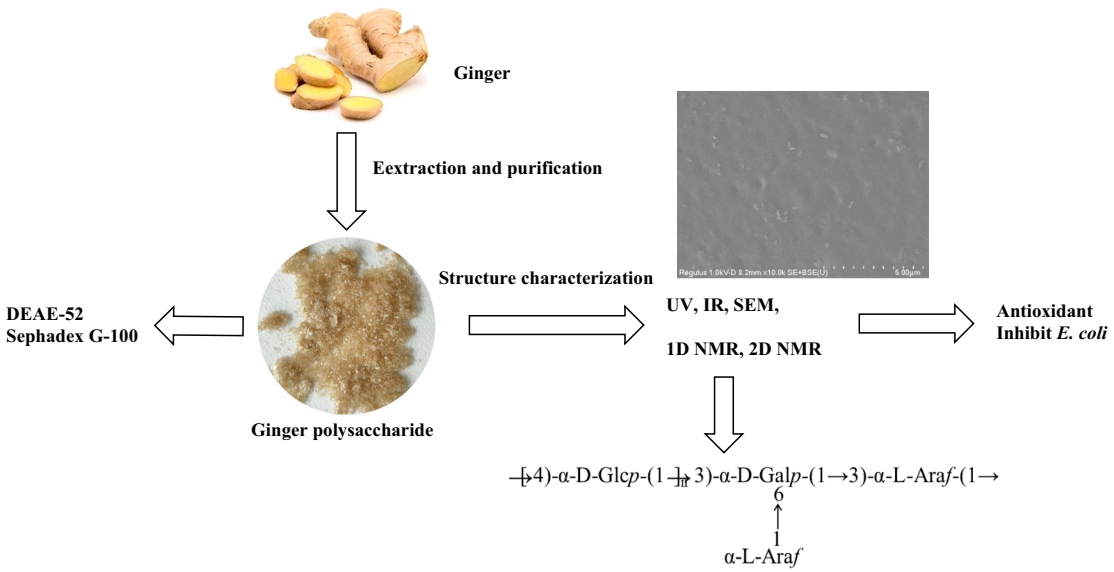
Graphical Abstract



Introduction

Polysaccharides are one of the most abundant substances in nature, which are used as a broad-spectrum immune regulator [1]. It can regulate the immune system (anti-tumor and antiviral efficacy), activate immune cells (strengthening the immune function of the body), and be combined with some drugs to prevent bone marrow suppression caused by chemotherapy. Ginger (*Zingiber officinale roscoe*) is a kind of fresh root of a perennial herb [2–4]. Ginger is a very valuable economic crop that is developed and used, except for physiologically active substances such as gingeroxone and gingerphenol, which also contains protein, polysaccharide, vitamins and a variety of trace elements, which is nutritious, seasoned, and healthy [5–7]. Since ancient times, the medical scientists have treated ginger as a health care product with the same origin in medicine and food, which has various health functions such as removing cold, removing wet, warming stomach and accelerating blood circulation [8]. Ginger products are becoming more and more popular in international markets, especially in countries such as southeast Asia and Japan. In China, the food and medicinal history of the ginger is very long, the development and utilization of which is also early. The content of which is low, but the main products were ginger, sweet ginger, ginger sauce, etc. To make good and reasonable use of precious ginger resources, through development and utilization, not only makes it fully utilized, but also obtains high value-added products, and obtains higher value-added effects [9–11]. Ginger polysaccharide is an important component of the ginger, which has various biological activities such as antioxidant, immune regulation, anti-tumor, anticoagulant blood and blood sugar [12, 13]. In addition, the spicy taste of ginger is mainly derived from the volatile oil and ginger ingredients, and a certain degree of upper limit was used to make the application of ginger polysaccharide [14]. In this study, the fresh ginger was used as the raw material, and the extraction using hot water to prepare the raw ginger polysaccharide, and then the new type of the ginger polysaccharide was obtained by the method of Sevage deproteinization, dialysis, DEAE-52 and Sephadex G-100. The chemical structure that may exist of the obtained ginger polysaccharide was characterized by UV, RI, SME, NMR (Fig. 1). On the basis of this, the derivation of the polysaccharide, the antioxidant activity, inhibition of *E. coli* were studied. This study could provide the theoretical and experimental bases for the biological activity of the ginger polysaccharide.

Materials and method

Materials and reagents

Fresh ginger (*Zingiber officinale roscoe*) was bought at Chongqing agricultural market in January 2023. After the identification of Professor Guangying Chen from Hainan Normal University, it was confirmed that it was

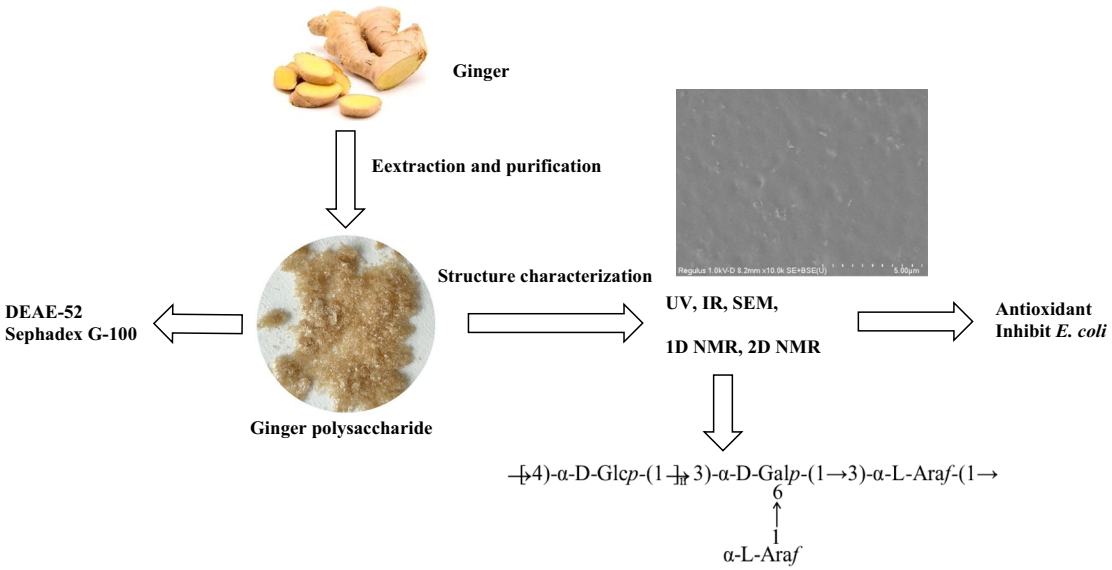


Fig. 1 Research content of ginger polysaccharide

the root part of ginger. The sample was stored in the Key Laboratory of Tropical Medicinal Plant Chemistry of Hainan Province, Hainan Normal University. All reagents used in the research were purchased from Aladdin (Shanghai, China).

Extraction of polysaccharide from ginger

Polysaccharide extraction process: fresh ginger → cleaning → drying → homogenate → polysaccharide extraction → centrifugation → collection supernatant → concentration → alcohol precipitation → centrifugation → collection precipitation → freeze drying → crude ginger polysaccharide.

The fresh ginger was washed and dried in the sun. The ginger was fully crushed by the homogenizer to obtain the ginger slurry. Weigh 100 g ginger grout and place it in a 1000-mL round-bottomed flask, add 500 mL distilled water according to 1:5 solid–liquid ratio, and fully stir it in a 90 ℃ magnetic stirrer for 3 h. After the polysaccharide extraction, the ginger was centrifuged for 10 min at 8000 r/min. The supernatant was collected, precipitated and treated twice, combined with ginger polysaccharide extract for 3 times. A rotary evaporator was used to concentrate the volume to 150 mL at 50 ℃, and a small amount of anhydrous ethanol (600 mL), equivalent to 4 times the volume of the concentrated solution, was added for 12 h at 4 ℃. Centrifugation (8000 r/min, 10 min), precipitation was collected, and the precipitation was freeze-dried to obtain crude ginger polysaccharide, weighing and calculating the yield. The yield of ginger polysaccharide was calculated by the following equation:

$$\text{Yield}\ (\%) = \frac{m_1}{m} \times 100\%.$$

m_1: The quality of the crude ginger polysaccharide (g).
m: The quality of ginger grout (g).

Refinement of the ginger polysaccharide

It was called the 5 g crude ginger polysaccharide, which was placed in the 500-mL round-bottomed flask. Which was added 300 mL of distilled water, and was fully stirred in the magnetic blender. Add the 100 mL Sevage reagent [chloroform: n-butanol = 4:1 (v/v)] to deprotein treatment of the crude polysaccharide. At room temperature, continue the magnetic stirring of 30 min and centrifugal at 8000 r/min. Collect the clear liquid, and the extract of the polysaccharide solution was processed with a protein. The deproteinized ginger polysaccharide solution was placed in a common dialysis bag (intercepted molecular weight 3500) and treated with tap water for 24 h and then with deionized water for 24 h. After the dialysis treatment, the ginger polysaccharide solution was concentrated. When the volume of the polysaccharide solution was concentrated to 50 mL, the 200 mL anhydrous ethanol was added, and the solution was left to stand at 4 ℃ for 12 h, centrifuged (8000 r/min, 10 min) to collect the precipitation, and the precipitation was freeze-dried to obtain the refined ginger polysaccharide.

The purification of the ginger polysaccharide

DEAE-cellulose 52 ion exchange column chromatography of ginger polysaccharide

The refined ginger polysaccharide was made up of 10 mg/mL solution, then add the DEAD-cellulose 52 ion exchange column (30×2.0 cm) in the area. In turn, the concentration of 0.0, 0.1, 0.2 and 0.3 mol/L NaCl solution was the graded eluent. The eluent rate was 36 mL/h, each tube 3 mL distribution collection, each gradient collection 20 tube, which was 60 mL per gradient. The content of polysaccharide was detected using phenol–sulfuric acid.

Sephadex-100 gel column chromatography of ginger polysaccharide

The purification of the ginger polysaccharide was isolated by using the Sephadex-100 gel tomography column (1.5×90 cm). The peristaltic pump was connected with the collector, and the gel was swelled, loaded and balanced for 24 h. Ginger polysaccharide was prepared with a concentration of 10 mg/mL, filtered by 0.45 μm microporous filter membrane, and then samples were taken with 0.2 mol/L NH_4HCO_3 as eluent. The condition of the eluent was 5 mL, velocity 0.3 mL/min, and each 10 min collects 1 tube. The content of polysaccharide was detected using phenol–sulfuric acid. After washing, the collected solution of the ginger polysaccharide solution was concentrated, dialysed and freeze-dried.

Preparation of polysaccharide derivatives from ginger

Preparation of phosphorylated polysaccharide

Weigh 5 g sodium tripolyphosphate and 1 g sodium trimetaphosphate into a small beaker and add 10 mL distilled water to dissolve thoroughly. To be accurate, the ginger polysaccharide of 0.5 g was placed in a 100-mL round-bottom flask, and the 20 mL of distilled water was stirred up to be fully dissolved. Phosphorylated reagent in the small beaker was slowly added to the round-bottom flask, and after the drop was finished, the pH value of the solution was 9.0 by 10% of the sodium hydroxide solution. Heat up to 70 ℃, continue to stir the polysaccharide for 5 h. After the phosphorolytic reaction was completed, the reaction liquid was cooled to room temperature, and it was placed in the common type dialysis bag (intercepted molecular weight was 3500), which was processed with water dialysis, 24 h, and the dialysis treatment of 24 h. Add 120 mL anhydrous ethanol to the polysaccharide solution after dialysis treatment. After resting at 4 ℃ for 12 h, centrifugation (8000 r/min, 10 min) was performed to collect precipitation and freeze-dried to obtain phosphorylated ginger polysaccharide (PGP).

Preparation of acetylated polysaccharide

Weigh 0.5 g of purified ginger polysaccharide and place it in a 100 mL round-bottomed flask. At room temperature, add 10 mL of distilled water to make a uniform polysaccharide solution. The NaOH solution of 10 percent of the solution was adjusted to pH 9.0, and continues to magnetic stir 20 min. Using the drip tube for 0.1 mL of acetic acid anhydride, the drop was waited for 2 min, and the pH was adjusted to 8.0–8.5 by 10 percent of the NaOH solution. So over 5 times, the drops were finished with all the acetic anhydride (0.6 mL). Heat the polysaccharide reaction fluid to 30 min, and continue the magnetic mixing reaction 30 min. After the reaction, use 2 mol/L HCl solution and pH to 7.0. The polysaccharide response fluid was placed in the common type dialysis bag (intercepted molecular weight was 3500), which was treated with water dialysis, 24 h, and the dialysis treatment of 24 h. The solution to the dialysis was added 100 mL anhydrous ethanol. After standing at 4 ℃ for 12 h, centrifuged (8000 r/min, 10 min) and the precipitate was collected; the polysaccharide was modified by acetylation after freezing and drying (AGP).

Determination of total sugar content

The total sugar content of the polysaccharide was determined by the method of phenol–sulfuric acid. The response system was 1 mL of glucose standard solution (0, 40, 80, 120, 160 and 200 μg/mL), 6% phenol solution of 0.5 and 2.5 mL of sulfuric acid. At room temperature, 40 min were left, and 100 μL of the samples were absorbed, and the OD value was measured at 490 nm wavelength. The concentration of polysaccharide samples was 100 μg/mL, the sample solution 1 mL, and measured the absorbance of the sample of the polysaccharide according to the standard curve.

Determination of degree of substitution

Determination of the degree of substitution of acetylated polysaccharides

Weigh 20 mg acetylated ginger polysaccharide, add 10 mL 0.01 mol/L NaOH solution to dissolve, heat to 50 ℃ and maintain 2 h, during the violent oscillation. Using phenolphthalein as an indicator, the red color disappeared by reverse titration with 0.01 mol/L HCl solution. The degree of substitution (DS) of acetylated ginger polysaccharide was calculated as follows:

$$DS = \frac{132A}{4300 - 42A}.$$

A: The acetyl content (%) in the sample.

Determination of degree of substitution of phosphorylated polysaccharide

10 mL of standard phosphorus solution with different concentrations were removed, followed by 4 mL of ammonium molybdate solution and 2 mL of 0.05 mol/L *Vc* solution. It was heated in a 100 ℃ water bath for 10 min, and then cooled to room temperature. The absorbance was measured at 824 nm with blank solution as the control. Pour 20 mg of phosphorylated ginger polysaccharide into the digestion bottle, add 1.5 mL of sulfuric acid–nitric acid solution [15], heat gradually, when the solution becomes clear, add 4.5 mL of distilled water, adjust the pH to 7, and treat according to the above phosphorus standard solution treatment method. The DS of phosphorylated ginger polysaccharide was calculated as follows:

$$DS = \frac{5.32M}{100 - 3.32M}.$$

M: The phosphorus content (%) in the sample.

Ultraviolet analysis and infrared analysis

The TU-1901 double beam ultraviolet–visible spectrophotometer was used to scan ginger polysaccharide in the wavelength range of 200–600 nm. Adopted the Fourier transform infrared spectrum instrument, and the infrared scanning of the ginger polysaccharide was carried out in the 4000–500 cm^{-1} wave to identify its main functional group.

Determination of monosaccharide components

Take a clean chromatographic bottle, accurately weigh 5 mg ginger polysaccharide sample, add 1 mL 2 mol/L trifluoroacetic acid solution. Heat up to 121 ℃ for 2 h. The ginger polysaccharide solution was dried with liquid nitrogen. Add methanol to clean, then blow dry, repeat methanol cleaning 2–3 times. Dissolve in sterile water and transfer to chromatographic bottle to be measured. Referring to the literature [21], the chromatographic system was Thermo ICS5000 ion chromatography system (USA), and the monosaccharide components were analyzed and detected by electrochemical detector.

Molecular weight determination

The ginger polysaccharide samples were dissolved in 0.1 mol/L $NaNO_3$ aqueous solution, and finally prepared into a polysaccharide solution with a concentration of 1 mg/mL, and filtered through a filter with a pore size of 0.45 μm. Referring to the literature [21], the molecular weight (*M*w and *M*n) of ginger polysaccharide was determined by gel chromatography, laser light scattering and differential detector.

Scanning electronic microscope analysis

The molecular morphologies of polysaccharides were observed using a scanning electronic microscope (Zeiss Merlin Compact, Germany). The samples, coated with a thin gold layer, were placed on the substrate, and the images were then observed at a voltage of 1.0 kV with 100- and 4000-fold magnification under high vacuum.

Methylation analysis

The sample was placed in a dryer at 80 ℃ for 5 h and then placed overnight in a vacuum dryer containing P_2O_5. After 18 mg of dried polysaccharide was placed in methylation reaction bottle, 4 mL of dried DMSO was added, the sample was completely dissolved by ultrasonic treatment for 30 min, and then 20 mg of pre-dried NaOH powder was rapidly added, and the NaOH powder was completely dissolved by ultrasound for 2 h, and the ice bath methylation reaction bottle for 5 min until the reaction was completely frozen. Take out the reaction bottle, slowly add 0.6 mL dry iodomethane with pipette until the frozen reaction is completely dissolved, fill with nitrogen, and then ultrasonic treat the reaction liquid for 1 h. Then 1 mL of distilled water was added to the reaction bottle to complete the methylation reaction. Then add 1 mol/L acetic acid neutralization solution. Water dialysis until the reaction solution turned colorless, freeze-drying, infrared detection of polysaccharide hydroxyl complete methylation. The fully methylated polysaccharide samples were dissolved in 4 mL 90% formic acid solution, and then depolymerized in a 110 ℃ oven for 6 h after packing. After the reaction, the reaction was dried under pressure, and 3 mL methanol was added to repeat the evaporation for 4 times to remove excess formic acid. The depolymerized samples were then added into a 4 mL 2 mol/L ampere-tube containing trifluoroacetic acid. After the tube was sealed and hydrolyzed at 110 ℃ for 2 h, the samples were then steamed under pressure and repeated with formic acid to remove excess trifluoroacetic acid. Add 3 mL distilled water to dissolve the sample. After 30 mg $NaBH_4$ reduction and acetylation, the partially methylated acetic acid derivatives were prepared. The derivatives were extracted by chloroform–water system, the chloroform layer was recovered, and the concentration was reduced to about 0.1 mL for GC–MS analysis.

Nuclear magnetic resonance analysis

1D-NMR and 2D-NMR (^{1}H-NMR, ^{13}C-NMR, COSY, NOESY, HMBC and HSQC) were recorded with a Bruker

AVANCE NEO spectrometer system (500 MHz) operated by Sanshu Biotech. Co., LTD (Shanghai, China).

The determination of ginger polysaccharide activity

Determination of ABTS + free radical scavenging rate

For reference [4], 7 mmol/L ABTS solution and 2.45 mmol/L potassium persulfate solution were prepared, and the two solutions were mixed evenly in equal volume, and placed at room temperature and away from light for 12 h to produce blue–green ABTS + free radicals. Before use, the ABTS working liquid was prepared by diluting the absorption value to 0.70 ± 0.02 (734 nm wavelength) with phosphate buffer solution (pH 7.4). 200 μL ABTS working liquid was mixed with 20 μL samples of different concentrations (0.1, 0.2, 0.4, 8, 1.6 and 3.2 mg/mL), and placed at room temperature for 6 min away from light. The light absorption value at 734 nm was determined. Distilled water was used as blank control instead of sample solution, and *V*c was used as positive control.

$$ABTS + free\ radical\ scavenging\ rate\ (\%) = \frac{1-(A_2-A_1)}{A_0} \times 100\%.$$

A_0: Water instead of the absorption value of the sample;
A_1: The absorption value of ABTS working liquid;
A_2: The absorption value of sample.

Determination of hydroxyl free radical scavenging rate

Reference [4] to the paper, mix 100 μL $FeSO_4$ solution (6 mmol/L), 100 μL sample solutions of different concentrations (0.1, 0.2, 0.4, 8, 1.6 and 3.2 mg/mL), and 100 μL H_2O_2 solution (6 mmol/L). After standing for 10 min, add 100 μL salicylic acid solution (6 mmol/L) and mix well. After 30 min of bath treatment, the absorption value of 510 nm was determined. The *V*c was used as positive control group, the distilled water was a blank control and three parallel measurements were measured.

$$Hydroxyl\ free\ radical\ scavenging\ rate\ (\%) = \frac{1-(A_2-A_1)}{A_0} \times 100\%.$$

A_0: Water instead of the absorption value of the sample;
A_1: The absorption value of H_2O_2;
A_2: The absorption value of sample.

Determination of DPPH radical scavenging rate

Weigh 5 mg DPPH reagent, add anhydrous ethanol to 100 mL, dissolve fully, and store away from light. The 0.1000 g polysaccharide samples were accurately weighed and prepared into aqueous solutions with concentration gradients of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/mL. 0.5 mL water solution of polysaccharide sample was added into the test tube, and 0.5 mL DPPH solution was added. After mixing, light absorption value was measured at 517 nm after reaction for 1 h. The Vc was used as positive control group and distilled water was used as blank control group.

$$DPPH\ radical\ scavenging\ rate\ (\%) = \frac{1-(A_2-A_1)}{A_0} \times 100\%.$$

A_0: Water instead of the absorption value of the sample;
A_1: The absorption value of DPPH;
A_2: The absorption value of sample.

Determination of reducing power

Accurately absorb 1.0 mL polysaccharide solution of different mass concentrations (0.1, 0.2, 0.4, 8, 1.6 and 3.2 mg/mL), add 2.5 mL phosphate buffer solution (pH 6.6 and 0.2 mol/L) and 2.5 mL 1% potassium ferricyanide solution, and react at 50 ℃for 20 min. Then 2.5 mL 10% trichloroacetic acid solution was added to stop the reaction. Centrifuge at 4000 r/min for 10 min, take 2.5 mL supernatant, add 2.5 mL anhydrous ethanol and 0.5 mL 0.1% $FeCl_3$ solution, mix, and measure absorbance at 700 nm wavelength. The *V*c was used as positive control group and distilled water was used as blank control group. The greater the absorbance measured, the stronger the reducing power of the sample.

Determination of inhibitory E. coli

An appropriate amount of *E. coli* (*Escherichia coli 1*) was inoculated in Luria–Bertani (LB) liquid base, and a polysaccharide solution of 100 μL with a concentration of 2 mg/mL was added. The culture solution was cultured at 37 ℃ at 120 r/min by shock. The absorbance of the culture solution was measured at 600 nm at an interval of 1 h. The growth curve of *E. coli* was determined by the same amount of inoculation, and the culture solution of the ginger polysaccharide. In addition, the amount of *E. coli* in the lb solid medium was 2 mg/mL of the ginger polysaccharide solution, and the 24 h on in the incubator of 37 ℃. The growth of *E. coli* was directly observed with the same amount of bacterial liquid and no polysaccharide as the control.

Statistical analysis

The SPSS20.0 software was used to analyze all test data, and the data results were expressed as mean ± standard

deviation. Statistical analysis was performed using one-way analysis of variance to determine the significance of the difference between the two groups. Set the significance level of the analysis results as probability less than 0.05 ($P < 0.05$).

Results and discussion

Extraction of ginger polysaccharide, preparation of its derivatives and determination of its substitution degree

In recent 10 years, the main methods used to extract ginger polysaccharide were hot water extraction, alkali solution extraction, microwave assisted extraction, ultrasonic assisted extraction, enzyme assisted extraction and so on. Hot water extraction was the traditional method of polysaccharide extraction. Water was used as the solvent to extract polysaccharide [3]. Through thermal action, plasma–wall separation occurs, in which osmotic pressure plays the main role. In this study, hot water was used to extract ginger polysaccharide, and the extraction conditions were optimized [4]. The extraction temperature was 90 ℃, and the extraction time was 3 h. Under the optimal technological conditions, the yield of crude ginger polysaccharide was 2.94% (calculated by the quality of fresh ginger). After extraction, ginger polysaccharide was mostly crude polysaccharide, which also contains many macromolecules and other impurities, which can have a certain impact on its subsequent research and production and application, so on this basis, it needs to be further refined. The removal of polysaccharides generally requires the steps of proteinization, dialysis and ethanol precipitation of crude polysaccharides. In the process of crude ginger polysaccharide refining, protein and nucleic acid were removed by Sevage method in this study, and the protein and nucleic acid in ginger polysaccharide solution would also interfere with the experimental results, so the extraction solution should be deproteinized. In the process of removing small molecules, dialysis was performed by intercepting dialysis bags with molecular weight greater than 3500. Finally, the refined ginger polysaccharide can be obtained after freeze-drying. In the preparation of ginger polysaccharide derivatives, acetylated and phosphorylated ginger polysaccharide were prepared in this study. The yields and substitution degrees of its derivatives were measured. As can be seen from Table 1, the yields of acetylation products in the ginger polysaccharide derivatives were relatively high, and the yields were more than double that of phosphorylated ginger polysaccharide. The yields of acetylation and phosphorylated products of ginger polysaccharide were 91.3 and 41.6%, respectively. The reason for the difference in yield of polysaccharide derived from ginger was that the preparation conditions are different. The acetylation reaction conditions were relatively mild, and the acetylation reagent used has high activity, while the phosphorylation reagent has low activity, which leads to the difference in yield of derivative preparation. The degree of substitution of polysaccharide derivatives has certain influence on its biological activity, so in this study, the degree of substitution of polysaccharide derivatives of ginger was determined. From the data in Table 1, it was found that the degree of substitution of acetylated ginger polysaccharide was high, while that of phosphorylated ginger polysaccharide was low, with the degrees of substitution of 0.87 and 0.39, respectively. It can be found from the substitution degree data that acetylation was relatively easy to prepare in the process of preparing ginger polysaccharide derivatives, but phosphorylation was difficult. Combined with the yield of ginger polysaccharide derivatives, it can be found that the yield and substitution degree are related in a certain sense. The phenol–sulfuric acid method was used to determine the total sugar content of ginger polysaccharide and its derivatives. As can be seen from Table 1, the total sugar content of ginger polysaccharide and its derivatives was relatively high, which were 93.5%, 92.1% and 87.2%, respectively.

Table 1 Determination of physical and chemical indexes of ginger polysaccharide and its derivatives

	Samples		
	GP	AGP	PGP
Yield (%)	2.94	91.3	41.6
DS	–	0.87	0.39
Sugar content (%)	93.5	92.1	87.2

Purification of ginger polysaccharide

After the polysaccharide was refined, it still needs to be further purified to obtain a purer polysaccharide of a single component as the basis for subsequent research, among which the purification and separation methods mainly include gel column chromatography and ion exchange chromatography [5]. In this study, after obtaining the refined ginger polysaccharide, gel column chromatography and ion exchange chromatography were used to further separate the ginger polysaccharide. When the polysaccharide was separated by DEAE-52 cellulose ion exchange column chromatography, different concentrations of NaCl were used as eluent, and the sugar content in the polysaccharide solution was tracked by phenol–sulfuric acid method. It can be seen from the separation diagram of DEAE-52 polysaccharide of ginger that polysaccharide of two components can be obtained

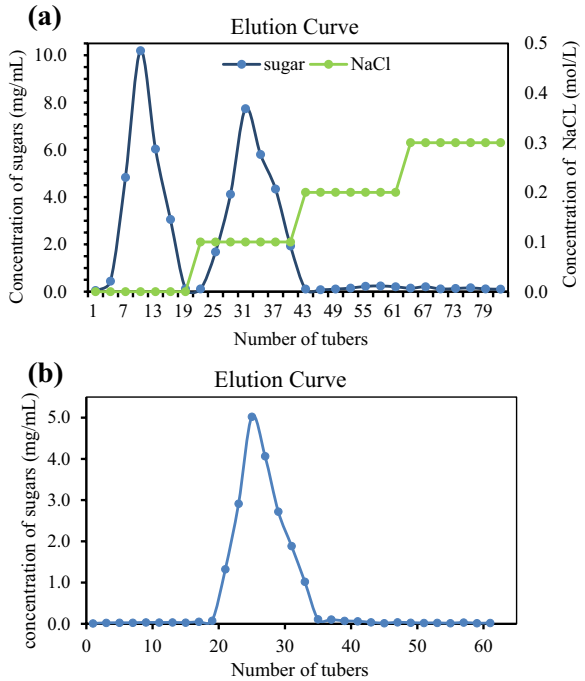


Fig. 2 DEAE-52 column chromatography elution curve of ginger polysaccharide (**a**). The gel purification elution curve of the sample was drawn with the number of eluent tubes as the horizontal coordinate and the total sugar content (blue) as the vertical coordinate. Sephadex G-100 column chromatography elution curve of ginger polysaccharide (**b**)

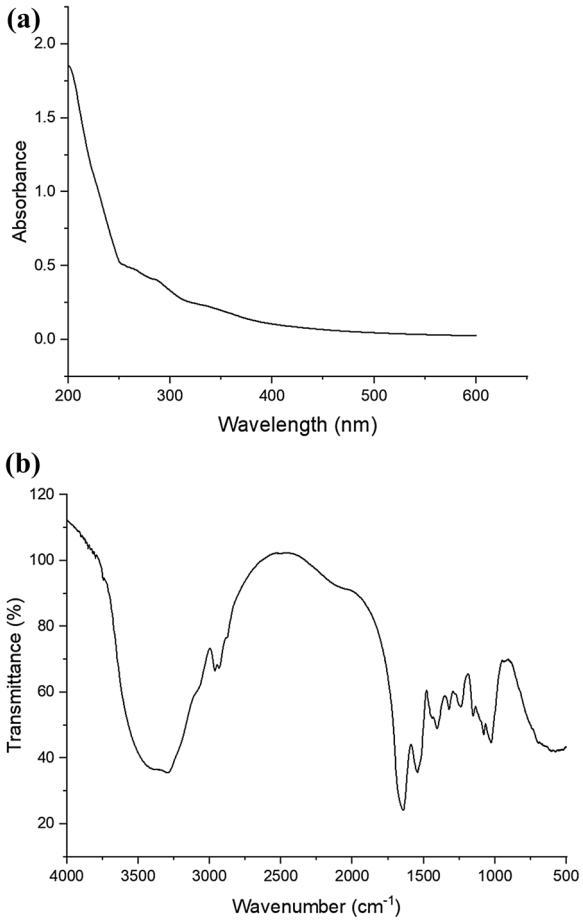


Fig. 3 Ultraviolet spectrum analysis of ginger polysaccharide (**a**); Infrared spectrum analysis of ginger polysaccharide (**b**)

(Fig. 2a). In order to obtain homogeneous components of ginger polysaccharide, Sephadex G-100 gel column chromatography was used to separate ginger polysaccharide. After the Sephadex G-100 gel column chromatography of ginger polysaccharide, it can be seen from Fig. 2b that there was only one set of peaks of ginger polysaccharide, which indicates that the purified ginger polysaccharide was a homogeneous polysaccharide. The results of total sugar determination showed that the purity of ginger polysaccharide was 94.2%.

Ultraviolet spectrum and infrared spectrum analysis of ginger polysaccharide

Full-wavelength scanning of the ultraviolet spectrophotometer can be used to determine whether the polysaccharide was completely removed from the protein and nucleic acid substances, usually these impurities have absorption peaks at 260 and 280 nm. Infrared spectroscopy can be used not only to determine the functional groups contained in polysaccharides, but also to identify the configuration of polysaccharides [22–25]. After purification, ginger polysaccharide was analyzed by ultraviolet spectrophotometer and infrared spectroscopy. It can be seen from the ultraviolet spectrogram (Fig. 3a) that after full wavelength scanning (200–600 nm), the absorption peak of ginger polysaccharide was not seen at 260 and 280 nm, so it can be inferred that the protein and nucleic acid substances in ginger polysaccharide have been cleaned. The infrared spectrum analysis of ginger polysaccharide showed that it had the general characteristics of polysaccharide in 4000–500 cm^{-1} region (Fig. 3b). There was no characteristic absorption peak of uronic acid in 1730 cm^{-1}, indicating that ginger polysaccharide may be neutral polysaccharide, and the structural characterization here was consistent with the results of monosaccharide determination. The wide and strong absorption peak between 3600 and 3300 cm^{-1} should be the O–H stretching vibration of sugar, the absorption peak between 3000 and 2800 cm^{-1} should be the stretching vibration of methylene C–H, and the set of peaks between 1400 and 1200 cm^{-1} should be the C–H variable angle vibration of sugar. From the above three points, it can be preliminarily determined that the group is divided

into sugars. The absorption peak at 844 cm^{-1} indicated that the glucoside bond in ginger polysaccharide was α-type [4]. There were 3 strong absorption peaks between 1200 and 1000 cm^{-1}, indicating that the sugar ring configuration of ginger polysaccharide was pyran type. The results of infrared spectrum analysis showed that the polysaccharide of ginger may be connected by α-type glucoside bond, and the sugar ring was a pyran ring.

Analysis of monosaccharide components, molecular weight and scanning electron microscopy of ginger polysaccharide

The chemical structure of polysaccharide was the basis of its biological activity, and the change of structure will affect its biological activity. Polysaccharide was a kind of complex biological macromolecule, and the complexity of its structure brings great difficulties to the process of polysaccharide resolution. The determination of monosaccharide composition and molecular weight of polysaccharides was an important work in the study of polysaccharide properties, which were often related to its monosaccharide composition and molecular weight. Three methods were commonly used to determine the composition of monosaccharides: high performance liquid chromatography (HPLC), gas chromatography (GC) and high performance anion exchange chromatography amperometric detection (HPAEC-PAD). There were two commonly used methods to determine molecular weight: high performance gel permeation chromatography (HPGPC) and gel chromatography-differential laser light scattering (GPC-RI-MALS). In this study, the monosaccharide composition of ginger polysaccharide was determined by HPAEC-PAD technique to achieve rapid qualitative and quantitative determination of monosaccharides. As can be seen from the ion chromatogram of ginger polysaccharide (Fig. 4a), its monosaccharide composition includes Fuc, Ara, Rha, Gal, Glc, Xyl, Man, Gal-UA, GLC-UA, and the percentage of each monosaccharide component was 1.01:1.96:9.54:14.82:64.20:3.84:2.07: 1.41:1.16. It can be seen from the monosaccharide composition data that ginger polysaccharide was a neutral heteropolysaccharide mainly composed of Rha (9.54%), Gal (14.82%) and Glc (64.20%). In the determination of molecular weight of ginger polysaccharide, the absolute molecular weight was determined by GPC-RI-MALS method. The molecular weight determination of ginger polysaccharide is shown in Fig. 4b, and its molecular weight was calculated to be M*w* = 35.52 kDa and M*n* = 216.56 kDa, and polydispersity index (M*w*/M*n*) was 1.64 [4]. Scanning electron microscopy (SEM) was the use of focused very narrow high-energy electron beam to scan the sample, through the interaction between the beam and the substance, to stimulate a variety of physical information, the information was collected, amplified, and then imaged to achieve the purpose of the microstructure characterization of the substance. SEM was a kind of observation means between transmission electron microscope and optical microscope, which was widely used to observe the surface ultrastructure of various solid substances (such as polysaccharides, nanomaterials, metals, etc.). In order to study the microstructure characteristics of ginger polysaccharide, SEM analysis was carried out. According to the SEM image (Fig. 4c), the main morphology of ginger polysaccharide between 20 and 5 μm showed a uniform dense lamellar structure, large and smooth fragments, no curling, and some of them showed the phenomenon of interlocking. This shows that the molecular weight of ginger polysaccharide was large, the morphology was uniform, and the intermolecular force was large [16].

Methylation analysis of ginger polysaccharide

Base analysis was one of the most powerful means to analyze the structure of polysaccharides and oligosaccharides. It includes all the free hydroxyl groups of sugar to form methyl ether, and then through hydrolysis to release methylated monosaccharides, and then reduced to sugar alcohols by $NaBH_4$, and then acetylated hydrolyzed hydroxyl groups to obtain a variety of partially methylated acetyl derivatives of sugar alcohols. Qualitative and quantitative analysis of the resulting products can be carried out by gas chromatography to determine the types and proportions of each monosaccharide composition. Then, by using gas chromatography–mass spectrometry combined with the analysis of standard spectra, we can obtain the identification of various partially methylated monosaccharide derivatives, so as to determine the location of the linkage of each monosaccharide, that was, the position of the glycoside bond [4]. In this study, methylation analysis of purified homogeneous component ginger polysaccharide shows that, as shown in Fig. 5 and Table 2, the 6 derivatives were detected, including 1, 4-di-O-acetyl-2, 3, 5-tri-O-methyl arabinito, 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl mannitol, 1, 3, 4-tri-O-acetyl-2, 5-di-O-methyl arabinitol, 1, 4, 5-tri-O-acetyl-2, 3, 6-tri-O-methyl glucitol, 1, 4, 5, 6-tetra-O-acetyl-2, 3-di-O-methyl glucitol and 1, 3, 5, 6-tetra-O-acetyl-2, 4-di-O-methyl galactitol. Therefore, it can be deduced that the directly existing bonding modes of each monosaccharide in ginger polysaccharide include t-Ara(f), t-Man(p), 3-Ara(f), 4-Glc (p), 4, 6-Glc (p) and 3, 6-gal (p).

1D and 2D analysis of ginger polysaccharide

One-dimensional hydrogen spectroscopy was mainly used to solve the problem of glycosidic bond configuration in polysaccharide structure [17–19]. Generally, the

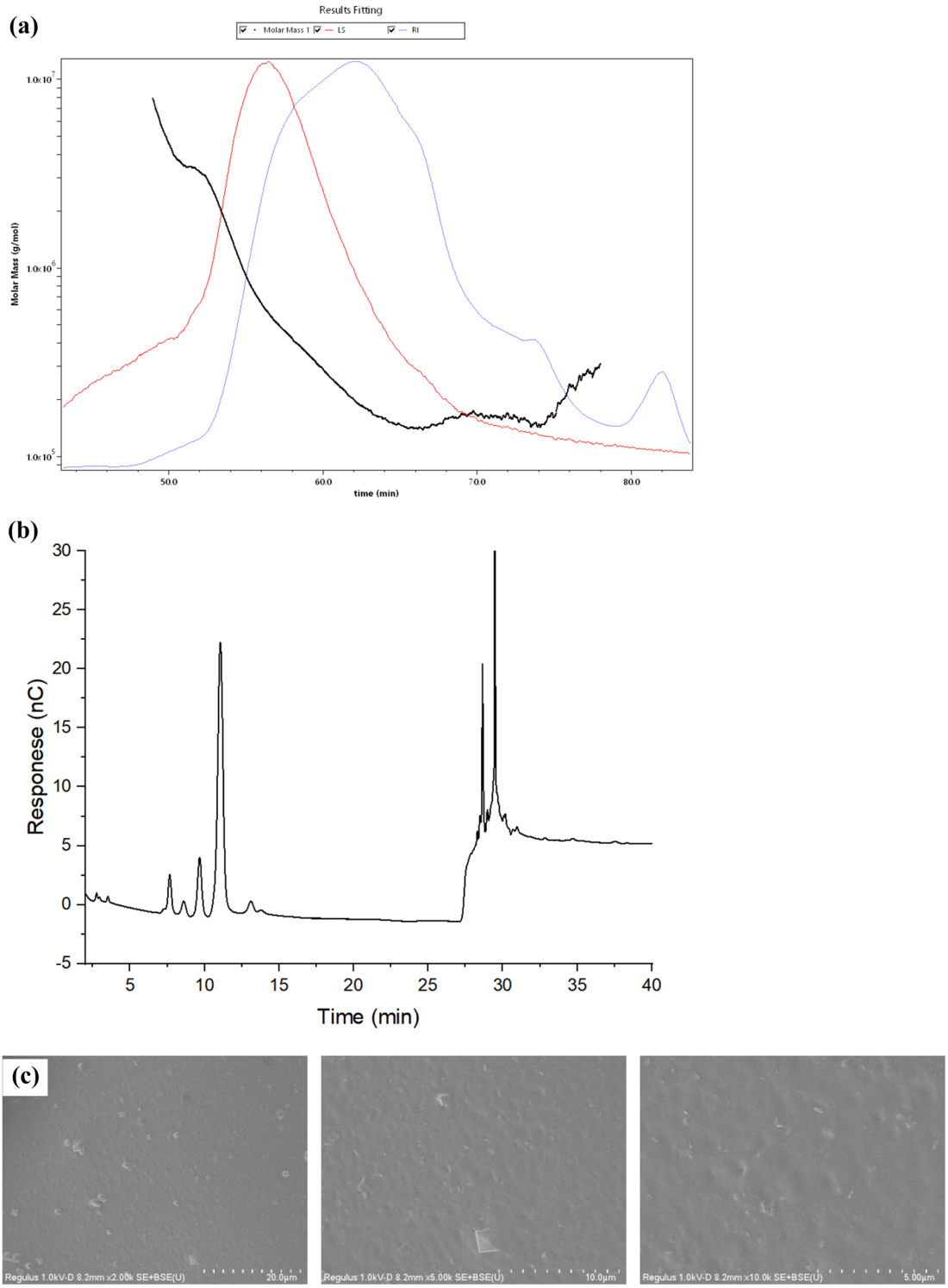


Fig. 4 Chromatograms for determination of molecular weight of ginger polysaccharide, red line: multi-angle laser light scattering signal; blue line: difference signal; black line: molecular weight fitted from the two signals (**a**). Ion chromatogram for determination of monosaccharide fraction of ginger polysaccharide. The mathematical relationship between the target monosaccharides and their peak areas was obtained by using the concentration of the standard monosaccharides as the horizontal coordinate and the peak area of the standard as the vertical coordinate. The content ratio of the target monosaccharides in the unknown ginger polysaccharide samples was calculated according to the peak area of the corresponding monosaccharides after complete hydrolysis (**b**). SEM of ginger polysaccharide, the surface structure of ginger polysaccharide samples was observed under the conditions of magnification to 2 k (20 μm), 5 k (10 μm) and 10 k (5 μm) (**c**)

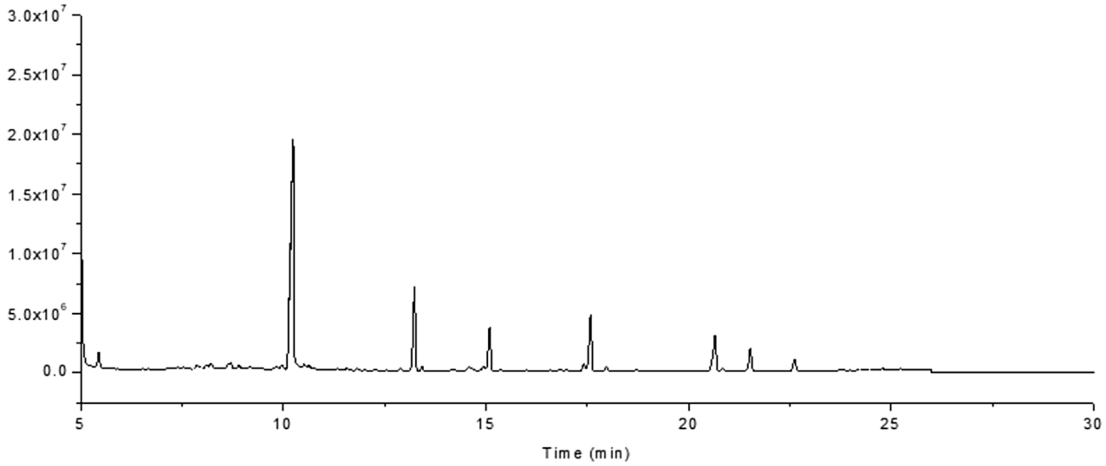


Fig. 5 Chromatogram of polysaccharide methylation analysis of ginger. After hydrolysis of ginger polysaccharide, under the combined action of electric field and magnetic field of mass analyzer, it was separated according to the size of m/z and then detected and recorded, so as to obtain the characteristic fragments of ginger polysaccharide after methylation

Table 2 Analysis of binding structure of ginger polysaccharide

Connection mode	Derivatives	RT	Mw	Relative molar ratio (%)
t-Ara(f)	1, 4-di-O-acetyl-2, 3, 5-tri-O-methyl arabinitol	5.441	279	13.237
t-Man(p)	1, 5-Di-O-acetyl-2, 3, 4, 6-tetra-O-methyl mannitol	8.219	323	5.201
3-Ara(f)	1, 3, 4-Tri-O-acetyl-2, 5-di-O-methyl arabinitol	8.7	307	5.323
4-Glc(p)	1, 4, 5-Tri-O-acetyl-2, 3, 6-tri-O-methyl glucitol	13.229	351	64.938
4,6-Glc(p)	1, 4, 5, 6-Tetra-O-acetyl-2, 3-di-O-methyl glucitol	17.417	379	5.020
3,6-Gal(p)	1, 3, 5, 6-Tetra-O-acetyl-2, 4-di-O-methyl galactitol	17.969	379	6.281

signal of plant polysaccharides in ^{1}H NMR was concentrated in δ 3–6 ppm. The allocephalic hydrogen signal of β-glycoside bond configuration is mainly distributed in δ 4.3–4.8 ppm, and the allocephalic hydrogen signal of α-glycoside bond configuration is mainly distributed in δ 4.8–5.8 ppm. As can be seen from Fig. 6a, the hydrogen spectrum signals of ginger polysaccharide are mainly concentrated in the δ 3.0–5.4 ppm range, and multiple coupling signal peaks are identified in the δ 4.3–5.4 ppm hetero-head signal region, indicating that ginger polysaccharide contains a variety of sugar residues. The corresponding chemical shifts of heterocephalic hydrogen are δ 5.32, 5.16, 4.55 and 4.41 ppm, respectively. The non-heterologous hydrogen signals are mainly concentrated in the δ 3.2–4.2 ppm region [20]. Due to the serious overlap of individual signals, the H2-H6 chemical shifts of each sugar residue need to be attributed according to COSY (Fig. 6c) and HSQC (Fig. 6e) spectra. The strong signal peak near δ 4.70 ppm was the solvent peak. Compared with ^{1}H NMR, the chemical shift signal distribution of ginger polysaccharide in ^{13}C NMR was wider. It can be seen from Fig. 6b that the heterocephalic carbon signal of ginger polysaccharide in ^{13}C NMR was concentrated in the 95–110 ppm range. Ginger polysaccharide can recognize multiple signal peaks in the heterotopic carbon region. Combined with the cross peaks of the heterotopic region of ^{13}C NMR spectrum and HSQC spectrum (Fig. 6e), the heterotopic signal in the polysaccharide was determined as follows: δ 5.32/99.62, δ 5.16/109.26, δ 4.41/103.02 and δ 5.02/107.56 ppm were denoted as sugar residues A, B, C and D, respectively. Based on the information of the bond structure (methylation) and the signal of the hetero-head of the sample and the comprehensive literature reports, it was inferred that the sugar residue A was →4)-α-D-Glc*p*-(1→, the residue B was α-L-Ara*f*-(1→), the residue C was →3, 6)-β-D-Gal*p*-(1→, and the residue D was →3) -α-L-Ara*f* -(1→. The ^{1}H and ^{13}C chemical shifts were attributed, and the results are shown in Table 3. According to the chemical shifts of ^{13}C and ^{1}H of each sugar residue in the sample, as the cross peak signal of HMBC spectrum (Fig. 6f) was relatively weak, combined with the NOESY

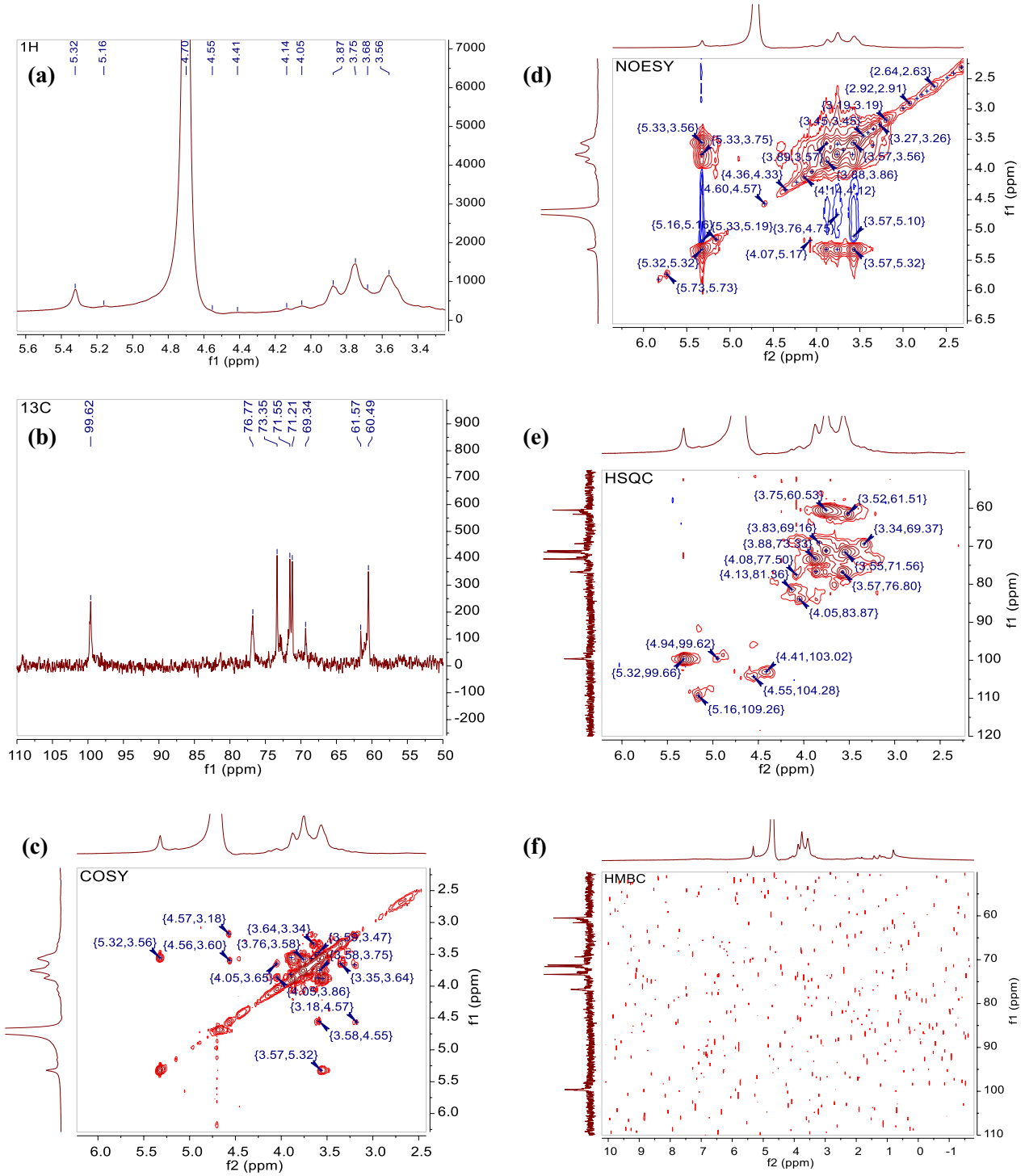


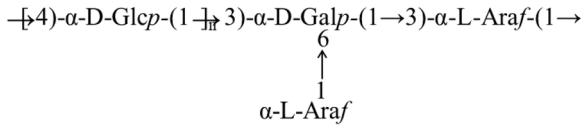
Fig. 6. ^{1}H NMR spectrum of ginger prunifolia polysaccharide (**a**). ^{13}C NMR spectrum of ginger polysaccharide (**b**). COSY spectrum of ginger polysaccharide (**c**). NOESY spectrum of ginger polysaccharide (**d**). HSQC spectrum of ginger polysaccharide (**e**). HMBC spectrum of ginger polysaccharide (**f**)

Table 3 The chemical displacement of ^{1}H and ^{13}C NMR of sugar residues

Code	Glycosyl residues	Chemical shifts (ppm)					
		H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6
A	→4)-α-D-Glc*p*-(1→	5.32	3.56	3.5	3.57	3.5	3.75
		99.62	72.6	73.35	76.8	70.38	60.53
B	α-L-Ara*f*-(1→	5.16	n.d	3.32	3.36	3.32	3.52
		109.26	n.d	72.31	70.91	71.06	61.51
C	→3, 6)-β-D-Gal*p*-(1→	4.41	n.d	3.87	3.88	3.55	3.83, 3.34
		103.02	n.d	76.66	69.3	69.73	69.37
D	→3)-α-L-Ara*f*-(1→	5.02	4.13	3.68	4.05	3.41	3.65
		107.56	81.36	80.13	83.87	75.72	60.93

n.d not detected

spectrum (Fig. 6d), the connection order of each residue in the polysaccharide was determined, and the cross peak δ 5.32/3.57 ppm existed between the sugar residue A-H1 and the residue A-H4. There was a cross peak δ 5.32/3.87 ppm with residue C-H3. There was a cross peak of δ 5.16/3.83 ppm between sugar residues B-H1 and C-H6. There was a cross peak of δ 4.41/3.68 ppm between sugar residues C-H1 and D-H3. Therefore, by combining 1D and 2D NMR information analysis, the possible structure of the polysaccharide main chain can be inferred as follows:



The structural representation of the polysaccharide derivatives

Polysaccharide was a kind of biological polymer widely existed in nature and has a wide range of pharmacological activities. However, not all natural polysaccharides have ideal biological activity [7]. The structure of polysaccharide determines its physiological function. Chemical modification of polysaccharide can improve its pharmacological activity and help to study the structure–activity relationship of polysaccharide. The analysis and study on the immune regulation of polysaccharide and its derivatives show that polysaccharide has antiviral, anti-tumor, immune-enhancing and anti-oxidation effects. The biological activity of polysaccharide was related to its structure, so the modification of polysaccharide structure has become the focus of polysaccharide research, and it was also an important way to discover and develop polysaccharide. The structural modification of polysaccharides refers to the appropriate modification of the molecular structure of polysaccharides by physical and chemical methods. Common chemical modification methods include sulfation, carboxymethylation, acetylation, phosphorylation, etc. In this study, the acetylated and phosphorylated ginger polysaccharides were prepared by chemical modification method with ginger polysaccharide as raw material, and their chemical structures were characterized. It can be seen from the ^{13}C NMR diagram of acetylated ginger polysaccharide (Fig. 7a) that compared with ginger polysaccharide, there was an obvious peak at the chemical shift of 175.00 ppm, where was the characteristic signal peak of carbonyl group (C=O). At the same time, there was a strong peak at the chemical shift of 43.86 ppm, where was the characteristic signal peak of methyl ($-CH_3$). Combining the peaks of the two chemical shifts, it can be inferred that the group was acetyl group, indicating that the ginger polysaccharide has been modified by acetyl group. After acetylation, the chemical shift of C1-C5 on the sugar ring was not obvious, and the chemical shift of C6 on the sugar chain was small. According to the ^{13}C NMR diagram of phosphorylated ginger polysaccharide (Fig. 7b), the chemical shift of C1-C5 and C6 on the sugar chain was smaller than that of ginger polysaccharide. ^{31}P NMR of phosphorylated ginger polysaccharide (Fig. 7c) showed that there were four peaks, indicating that four sites of ginger polysaccharide were modified by phosphate groups. It has obvious peaks at chemical shifts − 24.51 and − 25.07 ppm, which can be speculated that these two places were easily modified by phosphate groups.

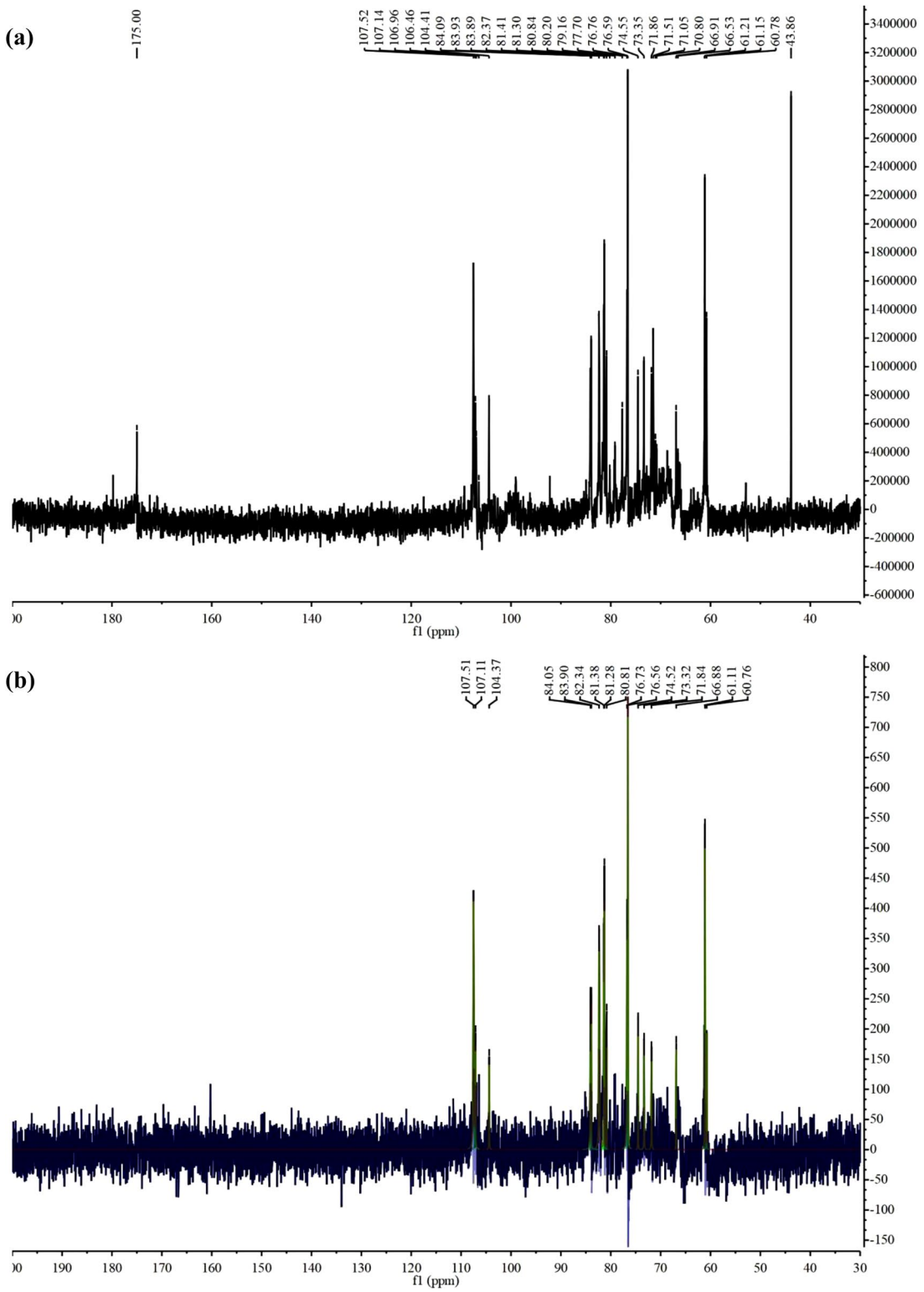


Fig. 7 ^{13}C NMR spectrum of acetylated ginger polysaccharide (**a**).^{13}C NMR spectrum of phosphorylated ginger polysaccharide (**b**). ^{31}P NMR spectrum of phosphorylated ginger polysaccharide (**c**)

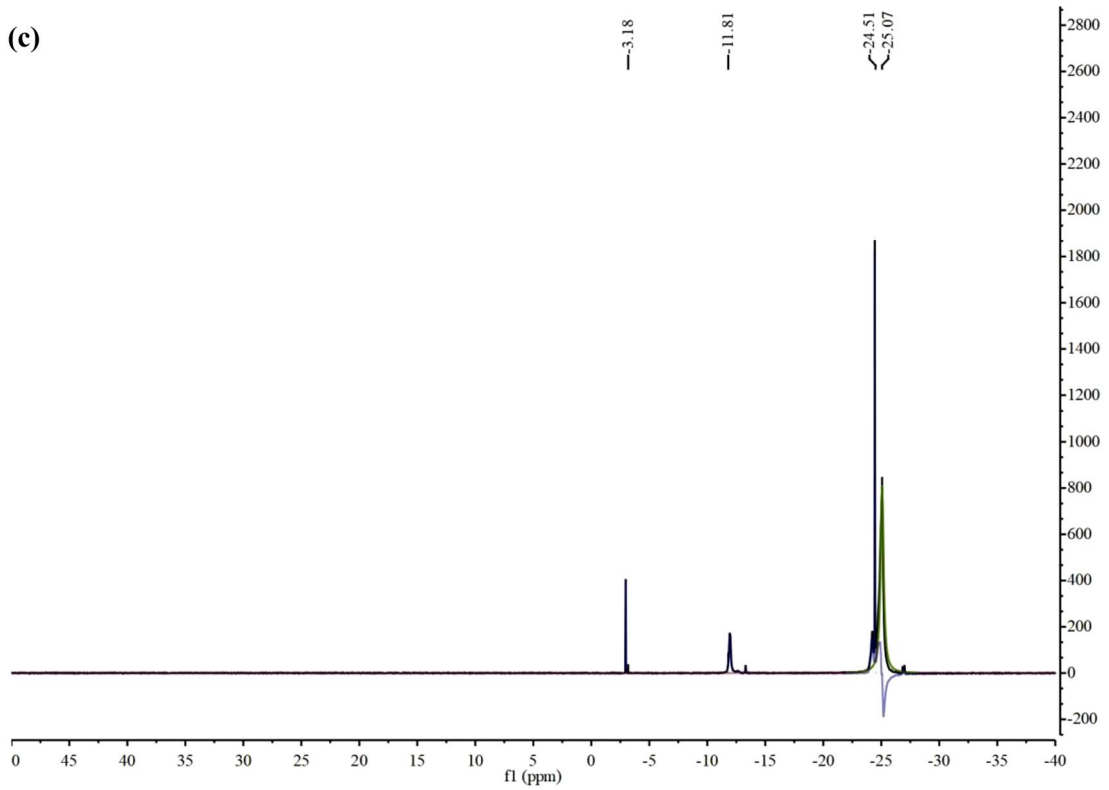


Fig. 7 continued

Biological activity of ginger polysaccharide and its derivatives

Anti-oxidant activity of ginger polysaccharide and its derivatives in vitro

Polysaccharides are a kind of natural polymer connected by aldose or ketose through glucoside bond. It was an important biological macromolecule in organism and one of the basic substances to maintain the normal operation of life activities. Nowadays, the research of plant polysaccharides has attracted more and more attention, and the international scientific community has even proposed that the twenty-first century was the century of polysaccharides. Scientific experimental studies have shown that many plant polysaccharides have biological activities, including immune regulation, anti-tumor, hypoglycemic, hypolipidemic, anti-oxidation, anti-radiation, anti-bacterial, anti-viral, liver protection and other health effects. Therefore, plant polysaccharides have long been widely used in public life fields such as medicine and catering. In this study, in order to study the antioxidant activity of ginger polysaccharide and its derivatives in vitro, we used ABTS + free radical clearance, hydroxyl free radical clearance, DPPH free radical clearance and reducing capacity as evaluation criteria [7]. As can be seen from Fig. 8a, vitamin C (Vc) has the best scavenging effect on ABTS + free radicals in the concentration range of 0.1–3.2 mg/mL. With the increase of concentration, the scavenging rate of ABTS + free radical was dose–effect relationship. When the concentration was 3.2 mg/mL, the scavenging rate of ABTS + free radical was 94.5% of ginger polysaccharide. Compared with ginger polysaccharide, the scavenging effect of phosphorylated ginger polysaccharide on ABTS + free radical was improved to a certain extent, and the clearance rate reached 98.3% at 3.2 mg/mL. In contrast, at the same concentration, acetylation of ginger polysaccharide did not significantly increase the scavenging rate of ABTS + free radicals. It can be seen from Fig. 8b that different concentrations of ginger polysaccharide and its derivatives can scavenge hydroxyl free radicals. In general, with the increase of polysaccharide concentration, the removal rate of hydroxyl radical has an obvious increasing trend. In addition, when the concentration was 3.2 mg/mL, the hydroxyl radical scavenging rate of ginger polysaccharide could reach 92.6%, and the phosphorylated ginger polysaccharide could reach 96.8%, which was close to the scavenging ability of *Vc* (97.5%). As can be seen from Fig. 8c, ginger polysaccharide and its derivatives have a good scavenging effect on DPPH free radicals,

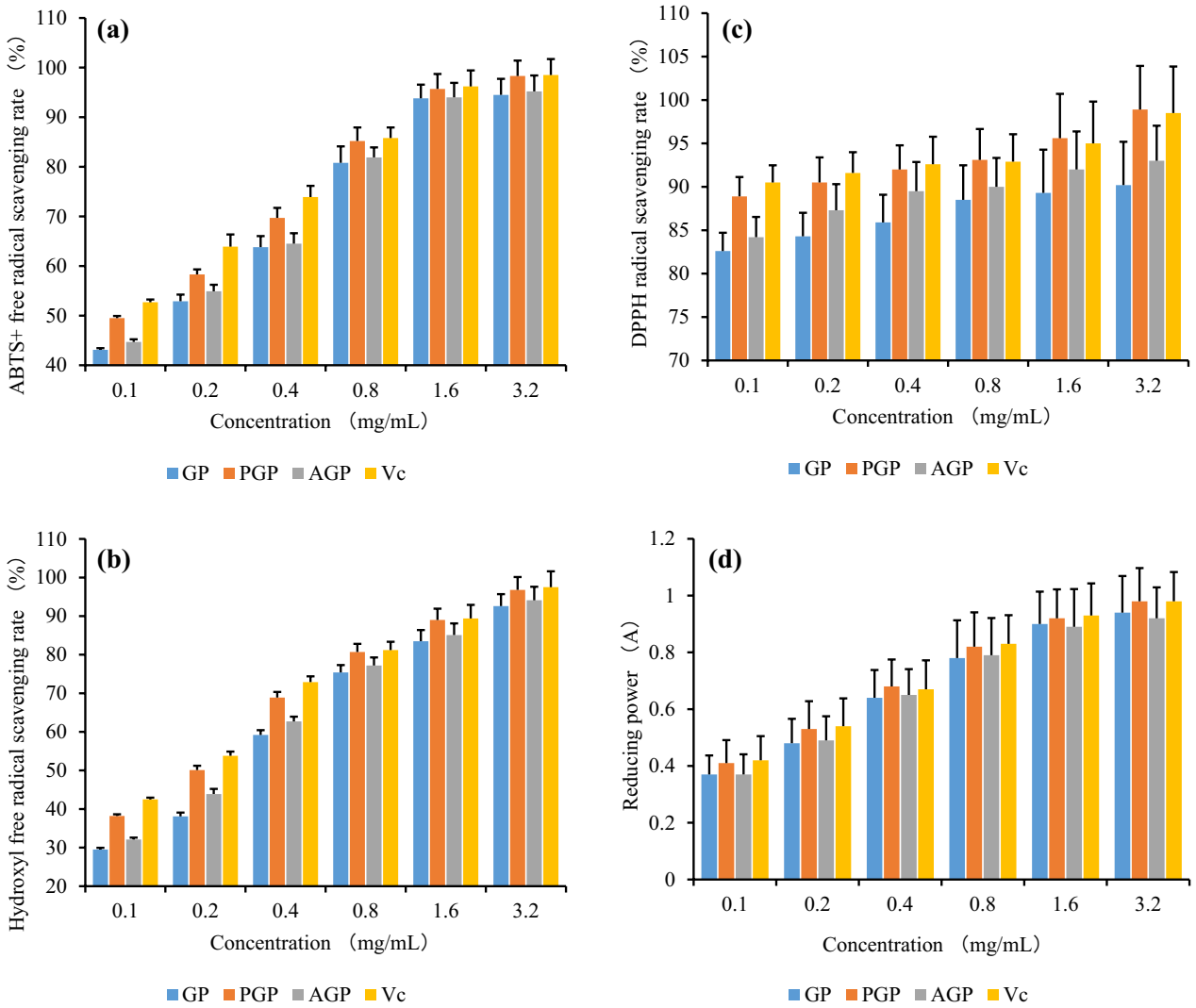


Fig. 8 ABTS + free radical scavenging rate of ginger polysaccharide and its derivatives (**a**). Hydroxyl radical scavenging rate of ginger polysaccharides and its derivatives (**b**). DPPH radical scavenging rate of ginger polysaccharide and its derivatives (**c**). Reducing power of ginger polysaccharide and its derivatives (**d**). In vitro antioxidant activity test, 6 gradient ginger polysaccharide solutions were selected to investigate the relationship between their concentration and activity. Each group of experiments was tested in parallel 3 times to find its average value and relative error value

but the influence of concentration was not particularly obvious. The derivatization of ginger polysaccharide can improve the scavenging ability of DPPH free radical, especially the phosphorylated ginger polysaccharide can reach or exceed the scavenging ability of *Vc* at all concentrations. It can be seen from Fig. 8d that ginger polysaccharide and its derivatives have good reducing ability, and the reducing power shows a significant concentration relationship between 0.1 and 3.2 mg/mL. When the maximum concentration of ginger polysaccharide was 3.2 mg/mL, the reducing power of ginger polysaccharide was close to that of *Vc*, and the reducing power of phosphorylated ginger polysaccharide was consistent with that of *Vc*. From the existing in vitro antioxidant activity data, ginger polysaccharide and its derivatives have a good biological activity. From the structure–activity relationship, the derived ginger polysaccharide can improve its activity to a certain extent, especially the phosphorylated ginger polysaccharide can basically reach the antioxidant level of the positive control *Vc* in terms of activity. This also indicates that phosphorylated ginger polysaccharide has the value of further study, including in vivo activity, mechanism of action, pharmacokinetics and so on.

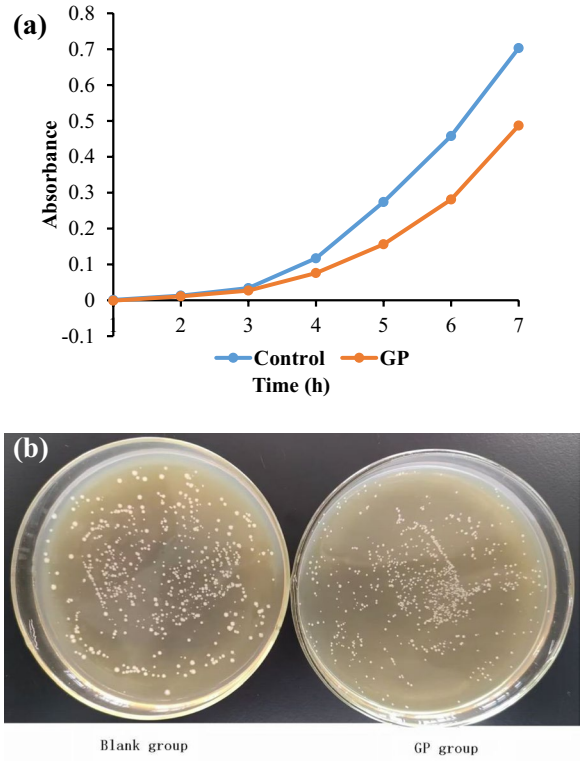


Fig.9 *E. coli* growth curve (**a**). *E. coli* culture (**b**)

Ginger polysaccharide inhibit E. coli in vitro

The results of liquid culture of ginger polysaccharide are shown in Fig. 9a, and the absorbance of polysaccharide sample and control increased with the extension of culture time. The difference of *E. coli* growth was small before 2 h, and then the difference between the two began to increase. However, after 6 h, the growth difference between the two began to shrink, because the higher concentration of bacteria in the control led to rapid nutrient consumption in the liquid medium, thus inhibiting the rapid growth of bacteria. However, the bacteria in the ginger polysaccharide sample made the absorbance difference between the two smaller because of the nutrients. According to the absorbance curve of bacterial growth, it can be concluded that ginger polysaccharide has the effect of delaying its growth. The results of liquid culture of begonia polysaccharide are shown in Fig. 9b. On the whole, the general bacterial colonies of control samples are larger than those of samples with polysaccharide, which further indicates that ginger polysaccharide has an inhibitory effect on *Escherichia coli*. However, after counting, there was no difference in the total number of colonies between the two, indicating that ginger polysaccharide had no bactericidal effect. These results may provide some insight into the HepG2 antiproliferative activities of ginger polysaccharide [2, 4].

Conclusion

In this study, the crude polysaccharide was extracted from the fresh ginger by water extraction and alcohol precipitation with a yield of 2.94%. The crude polysaccharide was deproteinized by Sevage method, small molecules were removed by dialysis, DEAE-52 ion exchange column and Sephadex G-100 gel column chromatography. The results of HPGPC analysis showed that the polysaccharide $M_w = 35.52$ kDa and $M_n = 216.56$ kDa, and the sugar content was 93.5%. Ion chromatography analysis to determine the ginger polysaccharide was mainly composed of Fuc, Ara, Rha, Gal, Glc, Xyl, Man, Gal-UA and Glc-UA, and the amount of substance ratio was 1.0 1:1.96:9.54:14.82:64.20:3.84:2.07: 1.41:1.16. Using methylation analysis, 1D and 2D NMR analysis, the results showed that the ginger polysaccharide mainly existed in A was →4)-α-D-Glc*p*-(1→, the residue B was α-L-Ara*f*-(1→), the residue C was →3, 6)-β-D-Gal*p*-(1→, and the residue D was →3) -α-L-Ara*f* -(1→. The bioactivity results of the ginger polysaccharide and its derivatives showed that they had good antioxidant bioactivity and the derivatization of the ginger polysaccharide could improve its activity to a certain extent, especially phosphorylated ginger polysaccharide which could basically reach the antioxidant level of positive control *Vc*. At the same time, the ginger polysaccharide also had a certain inhibitory activity against *E.coli*. This study not only had a certain guiding significance for the analysis of the structure and activity of the ginger polysaccharide, but also could promote the further study of the ginger polysaccharide and its derivatives.

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Author contributions
SZ, XW and WJ wrote the manuscript. SZ and GC reviewed and edited the manuscript. Both authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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