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Purification and structural characterization of Chinese yam polysaccharide and its activities

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ABSTRACT

Purification and structural characterization of Chinese yam polysaccharide were investigated and its activities were analyzed. Results indicated that a single component polysaccharide with a molecular weight of 16,619 Da was obtained after hot water extraction with sequential sevage deproteinization, HSCCC and Sephadex G-100 size-exclusion chromatography. The FTIR analysis showed that it had characteristic absorptive peaks and contained uronic acid. The methylation and GC–MS analysis showed that it comprised of glucose and galactose with a molar ratio of 1.52:1, and that it mainly contained 1,3-linked-glc, 1-linked-gal and 1,6-linked-gal glycosidic bonds. ^{1}H NMR and ^{13}C NMR spectra analysis showed that there were two α-configurations and one β-configuration, and that β-1,3-glucose, α-1-galactose, α-1,6-galactose might exist in the structure of the purified polysaccharide. The determination of the antioxidative activity showed that it could scavenge hydroxyl and superoxide radicals. The purified polysaccharide displayed a certain inhibitory activity against *Escherichia coli*, with a MIC of 2.5 mg/mL.

1. Introduction

Polysaccharides play an important role in the development of new products, including foods, pharmaceuticals, and biodegradable packaging materials (Chang, 2002; Cui, 2005; Jiao, Yu, Zhang, & Ewart, 2011; Kong et al., 2009; Srivastava & Kulshreshtha, 1989). The chemicals of low molecular weights, such as carotenoids, tocopherols, ascorbic acid and flavonoids, etc., are closely related to the human health (Halliwell, Rafter, & Jenner, 2005; Middleton, Kandaswami, & Theoharides, 2000; Riederer et al., 1989; Robak & Gryglewski, 1988; Roe & Kuether, 1943; Seddon et al., 1994; Ziegler, 1989). Polysaccharides are renewable sources for the sustainable supply of cheaper pharmaceutical products (Srivastava & Kulshreshtha, 1989). In recent twenty years, the development of new bioactive polysaccharides for functional foods or pharmaceuticals, has emerged as one of the hot topics in chemistry- and biology-related industries.

Chinese Yam, namely *Rhizoma Dioscoreas Oppositae*, is one of the well-known edible and pharmaceutical foods in China, and mainly contains polysaccharides, flavonoids, allantoin, trace elements and other active ingredients (Wang, Yu, Gao, Liu, & Xiao, 2006; Zhao, Kan, Li, & Chen, 2005; Zhao, Li, & Chen, 2002). Previous studies have shown that Chinese yam extracts have a strong anti-oxidative capacity and a high comprehensive utilization value, which can be used to effectively remove free radicals (Jeon et al., 2006). Studies on Chinese yam polysaccharides have mainly focused on the crude extraction and their activities analysis (Zhao et al., 2005, 2002), while the investigation of their structural characterizations is very little. Because of the large number of varieties and different growth conditions of Chinese yam, there are big differences on their structures and bioactivities. In terms of activities, many studies have confirmed that Chinese yam polysaccharides can enhance immunity and lower blood sugar, and have pharmacological functions (Chen, Wong, Zheng, Bai, & Huang, 2008; Zhao et al., 2005, 2002). Thus, it is interesting to purify Chinese yam polysaccharide to analyze and characterize its structures, which can be used as a potential antioxidant and an antibacterial pharmaceutical, and can provide theoretical basis for the future use in related fields.

In this study, the hot water extraction was performed to separate Chinese yam polysaccharide. The separated polysaccharides were further purified by sevage deproteinization with sequential high-speed countercurrent chromatography (HSCCC) and Sephadex G-100 size-exclusion chromatography. Fourier transform infrared spectroscopy (FTIR) was used to analyze the absorptive peaks of the purified polysaccharides and the interaction among molecules. Gas chromatography-mass spectrometry (GC–MS) and nuclear magnetic resonance (NMR) were used to characterize the structures of purified polysaccharides. Moreover, Chinese yam polysaccharide

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activities, including the anti-oxidative and antibacterial activities, were also investigated.

2. Materials and methods

2.1. Materials

Chinese yam was purchased from the Lianhua commercial market, Hangzhou, Zhejiang Province, China. Glucan and the other standard samples were purchased from the Sigma Co. Ltd, USA. Unless otherwise stated, all chemicals used were of analytical grade, and were purchased from the Huipu Chemical Co, Ltd, Hangzhou, Zhejiang Province, China.

2.2. Chinese yam pretreatment

Selected yams were washed thoroughly with water, and were cut into small pieces. The pieces were dried with 1.5% citric acid, 0.75% sodium sulfate, and 1.5% sodium chloride in a tray dryer at 50 °C for 72 h to obtain dry chips, which were then ground in a high speed disintegrator to obtain the dry Chinese yam powder.

2.3. Hot water extraction

The effect of pH, temperature, extraction time and the ratio of water to yam powder on the yield of Chinese yam polysaccharide was studied by the single factor experimental design. One factor is changed, while the other factors are kept constant in each experiment. The effect of each factor was evaluated by determining the yield of the yam polysaccharide.

2.4. Determination of the Chinese yam polysaccharide yield

After the hot water extraction, the slurry obtained under the optimal conditions was centrifuged at 8000 rpm for 15 min to collect the supernatant. The supernatant was mixed with 3-fold volume of absolute ethanol, and was then kept for 2 h. The solution was centrifuged to obtain the precipitate. The precipitate was diluted with water, and was then concentrated using a rotary evaporator at 40 °C under the vacuum condition to remove absolute ethanol. It was then dried in a vacuum drier until its weight was constant, and was weighed with a balance (AY120, Shimadzu, Japan). The yield of yam polysaccharide (%) was calculated as given below (Yu & Chao, 2013):

$$\text{polysaccharide yield}\,(\%) = \frac{A}{B} \times 100\%$$

where A and B are the weight of Chinese yam polysaccharide (g) and Chinese yam powder (g), respectively.

2.5. Deproteinization and HSCCC purification

The sevage method was used for deproteinization from Chinese yam polysaccharide (Huang, Yang, & Wang, 2010), and the times of deproteinization was determined. After that, HSCCC was used for purifying Chinese yam polysaccharide as described by Yu and Sun (2014) except that kelp crude polysaccharide was replaced by Chinese yam crude polysaccharide. The solvent system for the HSCCC separation was selected by determining the partition coefficient of a series of solvent systems composed of different proportions of PEG1000–K_2HPO_4–KH_2PO_4–H_2O. The solvent system composed of PEG1000–K_2HPO_4–KH_2PO_4–H_2O with a ratio of 12.5:8:8:71.5 (w/w) was finally chosen for HSCCC. In this solvent system, the upper phase was viewed as the stationary phase, and the other phase as the mobile phase. When the stationary phase was packed fully, the mobile phase was pumped into the inlet of the column with a flow rate of 2.5 mL/min. The apparatus was started at 700 rpm. 200 mg of Chinese crude polysaccharide dissolved in 20 mL of the lower phase was injected into the HSCCC system until the mobile phase was outflowed from the outlet of the column. 8 mL of each effluent was collected by a collector, and the phenol–sulfuric acid colorimetric method was used to determine the distribution of Chinese yam polysaccharide (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The same polysaccharide components were combined, and the dialysis bag with a molecular weight cut-off of 3500 Da was used to remove small molecular substances and PEG1000. The samples were dried in a vacuum freeze drier until their weight was constant, and were then kept at 4 °C until use.

2.6. Sephadex G-100 purification and HPLC–GPC analysis

9 mg of Chinese yam polysaccharide was dissolved in 3 mL of the distilled water, and was loaded onto a Sephadex G-100 column (1.0 × 100 cm). The column was eluted by the distilled water at a flow rate of 0.2 mL/min. 3 mL of each effluent was collected by a collector, and the content of polysaccharide in the effluent was detected by the ultraviolet and visible spectrophotometer as described by Dubois et al. (1956). The same polysaccharide components were combined, and were dried in a vacuum freeze drier until their weight was constant. The gel-permeation chromatography fixed with a TSK-G4000PWxL column was used to identify if the polysaccharide was pure, and to determine its average molecular weight.

2.7. FTIR analysis

The pure polysaccharide was dried in a vacuum freeze drier with P_2O_5 for several days to remove water. About 1 mg of the solid polysaccharide was mixed with 100 mg of KBr under the anhydrous condition, and this mixture was then analyzed and scanned on the FTIR spectroscopy to record FTIR spectrum in the range of 400–4000 cm^{-1}.

2.8. Acetylation of monosaccharide standard samples and GC–MS analysis

Seven standard monosaccharide samples, including galactose, fructose, xylose, rhamnose, glucose, mannose and arabinose, were respectively dissolved in a 10 mg/mL of sodium borohydride ($NaBH_4$) solution. After that, glacial acetic acid was added to neutralize the excess $NaBH_4$ until no bubbles appeared. 3 mL methanol was added, and the solution was evaporated to dryness when the pH of the mixture was 4–5. Repeat the above steps for 4 to 5 times to remove reactive byproducts, and then put the sample into a vacuum freeze dryer overnight. The sample was heated in the oven at 100 °C for 15 min to remove the residual water fully, and then 4 mL acetic anhydride was added. 3 mL toluene was added after the sample was cooled. The sample was dried under the reduced pressure condition. Repeat above steps for 4 to 5 times to remove the excess acetic anhydride. The acetylated product was dissolved into 3 mL chloroform, and the solution was then transferred to the separator funnel with a small amount of the distilled water. The mixture was shaken fully to remove the upper solution. Repeat above steps for 4 times until the upper solution was removed fully. The chloroform layer was dried with an appropriate amount of anhydrous sodium sulfate for the GC–MS analysis.

2 mg of Chinese yam polysaccharide sample was hydrolyzed with 4 mL of 2 mol/L trifluoroactic acid (TFA) at 110 °C for 2 h. After being removed the residual TFA under the reduced pressure condition, the hydrolyzed sample was mixed with 3 mL methanol. Repeat above steps for 4 to 5 times to remove TFA completely.

After acetylation, chloroform was added to the sample till a volume of 5 mL. Derivatives were analyzed by GC–MS using a DB-SMS quartz capillary column (30 m × 0.25 mm × 0.25 μm). Column initial temperature 80 °C, keep 1 min; ramp rate 5 °C/min, keep 1 min to 185 °C; ramp rate 2 °C/min, keep 1 min to 202 °C; ramp rate 10 °C/min, keep 1 min to 270 °C. Carrier gas was He, the split ratio was 1:10. The ion source was EI, ionization energy was 70 eV and the filament current was 250 μA. The temperature of the ion source and the GC–MS interface was 230 °C and 250 °C, respectively. The mass scanning scope was from 42 to 462 amu, and scan rate was 2.5 scan/s. Several standard monosaccharide samples were used as the control under the same conditions.

2.9. Methylation and acetylation analysis

0.5 g NaOH was dissolved into 1 mL distilled water. 0.2 mL NaOH was mixed with 0.2 mL methanol. The mixture was diluted with 6 mL dimethyl sulphoxide (DMSO), and was then shaken on the vortex mixer. After that, the suspension was put into the ultrasonic bath for 5 min, and was centrifuged to collect NaOH precipitation. 2 mg sample and 0.5 mL DMSO were put into the ultrasonic bath for 2 min, and were then standed at 25 °C for 30 min. 0.6 mL NaOH–DMSO suspension and 0.6 mL methyl iodide were added to the sample. The sample was shaken on the vortex mixer for 7 min. After that, the methylation was terminated by adding 4 mL H_2O. The sample was extracted using the same amount of chloroform. The organic phase was concentrated under the reduced pressure condition, and the obtained sample was analyzed by the infrared spectrum detection. Repeat above operations until the methylation was completely analyzed. Methylated samples were dissolved in a 3 mL of 88% formic acid solution, and were plugged and depolymerized at 100 °C for 3 h. 3 mL methanol was then added, and the solution was evaporated to dryness at 40 °C. Repeat above steps for 3 times. 4 mL of 2 mol/L TFA was added to depolymerized polysaccharide samples, which were then hydrolyzed at 100 °C for 6 h. The sample solution was evaporated to dryness at 40 °C. Repeat above steps for 5 times, and add 20 mg $NaBH_4$ to the sample. The sample was dissolved into 3 mL of the distilled water. The acetylation process of the sample was the same as that of the monosaccharide standard sample.

2.10. NMR spectroscopy

^1H NMR and ^{13}C NMR spectra of the purified polysaccharide were recorded in an NMR apparatus. 30 mg of the purified polysaccharide was dissolved in deuterium oxide, and was then dried at a vacuum freeze drier.

2.11. Hydroxyl radical scavenging

The hydroxyl radical scavenging activity was investigated as described by Yang et al. (2014) with minor modification. The polysaccharide sample was dissolved in 10 mL of distilled water at the concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. 1.0 mL of the sample solution was mixed with 1.0 mL of 8.8 mmol H_2O_2, 1.0 mL of 9 mmol $FeSO_4$, and 1.0 mL of 9 mmol/L ethanol salicylate. The reaction solution was incubated at 37 °C for 30 min. The absorptive value of the reaction solution was detected at 510 nm using the distilled water as the control. Vitamin C was used as a positive control. The scavenging capability to hydroxyl radical was calculated by the following equation:

$$\text{scavenging effect}\,(\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

A_0—the absorptive value of the control samples; A_1—the absorptive value of the samples.

2.12. Superoxide radical scavenging

The superoxide radical scavenging activity was investigated as described by Nandi and Chatterjee (1987) with minor modification. Samples were dissolved in the distilled water at 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL. An aliquot of each sample solution was mixed with a 6.0 mL of 50 mmol Tris–HCl buffer solution (pH 8.2) and 2 mL of the distilled water. The mixture was shaken and kept in water bath at 37 °C for 10 min. The sample was then mixed with a 1.0 mL of 3.5 mmol/L phthalate triphenol solution, which was preheated in a water bath at 37 °C. After 6 min, the reaction system was quickly terminated by the addition of 0.5 mL HCl (8 mmol). The absorptive value at 420 nm was determined. Vitamin C was used as a positive control. The superoxide radical scavenging capacity (%) was calculated as follows:

$$\text{scavenging effect}\,(\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

A_0—the absorptive value of the control samples; A_1—the absorptive value of the samples.

2.13. Antibacterial activity analysis

Selected strains, including *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Nissl bacillus*, were used for testing the antibacterial activity of the purified yam polysaccharide. 1 mL of the cell suspension (10^8 CFU/mL) was inoculated in a 200 mL Luria-Bertani (LB) culture broth, and was then shaken to make them mixed adequately. The mixture was spread on the solid LB petri plate before solidification (Zhang et al., 2013). Sterilized oxford cups with a diameter of 8 mm were standed on the petri plate, and 200 mL of Chinese yam polysaccharide samples with different concentrations were respectively added into oxford cups. The sterilized water was added into the oxford cup as the control. The plates were incubated at 37 °C for 12 h. The presence of the inhibitory zone around the oxford cup indicated that the purified yam polysaccharide had an antibacterial activity. The tests were performed in triple times, and the average size of the inhibitory zone was presented (Wang, 2014). The minimal inhibitory concentration (MIC) for selected strains was regarded as the lowest concentration which presented an inhibitory zone.

3. Results and discussion

3.1. Hot water extraction

With the increase in pH, the yield of Chinese yam polysaccharide showed a trend of increase and then decrease (Fig. 1a), and reached a maximum at pH 9.0. However, it significantly became lower when pH was above 9.0. It was speculated that a high pH affected the stability of Chinese yam polysaccharide, or that the solubility of Chinese yam polysaccharide decreased in the alkaline solution. Fig. 1b showed the relationship between the extraction time and the yield of Chinese yam polysaccharide. At 1.25–1.5 h, it was clear that the increase in the yield of Chinese yam polysaccharide was obvious with the increase in the extraction time. At 1.5–1.75 h, the yield of Chinese yam polysaccharide declined. It was probable that Chinese yam was gelatinized as the time prolonging, which was not good for separation. When the extraction temperature varied from 50 to 100 °C, the yield of Chinese yam polysaccharide increased (Fig. 1c), especially within the range of 70–80 °C. It is probable that higher extraction temperature is good for facilitated diffusion which leads to the leaching of Chinese yam polysaccharide. However, the yield of Chinese yam polysaccharide declined significantly when the temperature was higher than 80 °C. On the other hand, the high temperature might contribute

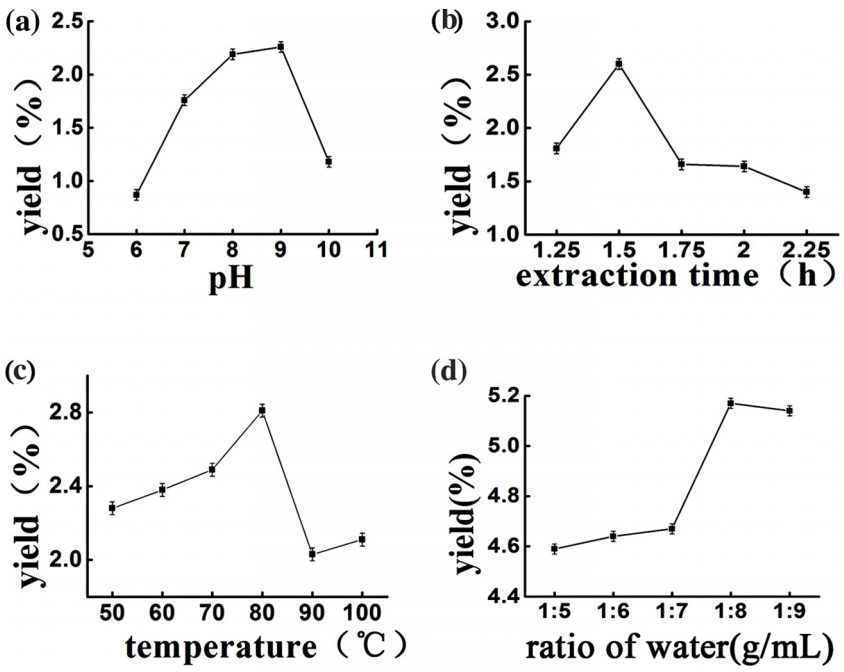

Fig. 1. Effect of pH, extraction time, temperature, and the ratio of water to yam powder on the yield of Chinese yam polysaccharide. (a) pH, (b) extraction time, (c) temperature and (d) the ratio of water to yam powder.

to the partial degradation of Chinese yam polysaccharides. Higher temperature made the liquid evaporate faster, which was unbeneficial to the extraction of the polysaccharide. The ratio of water to yam powder showed a positive effect on the yield of Chinese yam polysaccharide (Fig. 1d). The ratio of water to yam powder apparently affected the extraction yield whether or not this ratio was very less or high (Balavigneswaran, Sujin Jeba Kumar, Moses Packiaraj, Veeraraj, & Prakash, 2013). The yield of Chinese yam polysaccharide reached a maximum at the ratio of water to yam polysaccharide 1:8. When there was little water, it was too sticky to undergo the solvent diffusion of polysaccharide, and this led to lower the yield of Chinese yam polysaccharide. Taken together, the highest yield of Chinese yam polysaccharide was 5.19% under the optimal conditions obtained by single factor experiments: pH 9.0, extract time 1.5 h, temperature 80 °C, and the ratio of water to yam powder 1:8.

3.2. Sevage deproteinization and purification of yam polysaccharide

As shown in Fig. 2a, with the increase in the deproteinization times, the loss rate of Chinese yam polysaccharide increased, but the content of protein decreased gradually. The content of protein kept constant when deproteinization times varied from 6 to 7, but the loss rate of Chinese yam polysaccharide still increased. The results indicated that 6 times of deproteinization was appropriate and the protein content was 5.91%. As shown in Fig. 2b, two components of Chinese yam polysaccharide were obtained by HSCCC. The second component was difficult to collect due to a low yield. Fractions of polysaccharide components (45–65 tubes) were combined and dialyzed. After the small molecular substances and PEG1000 were removed, collected fractions were evaporated under the freeze-dried condition to obtain Chinese yam polysaccharide. As shown in Fig. 2c, the profile of Chinese yam polysaccharide detected by the phenol–sulfuric acid colourimetric method showed that it was the same component with a symmetric peak after the purification by Sephadex G-100. Then, the same fractions were collected (10–25 tubes), and was dried under the freeze-dried condition. As shown in Fig. 2d, the GPC-HPLC profile of Chinese yam polysaccharide presented a single symmetrical peak with a retention time of 11.723 min. The standard curve correlated the molecular weight with the GPC-HPLC retention time of the standard samples has the following formula: $\log(M_w) = -0.5365Rt + 10.51$. In this formula, M_w is the molecular weight of glucan, and Rt is the retention time. Thus, the molecular weight of the purified Chinese yam polysaccharide should be 16,619 Da.

3.3. FTIR analysis

As shown in Fig. 2e, there were typical absorptive peaks of glycosidic structures in Chinese yam polysaccharide. The strong absorptive peak of 3426.93 cm^{-1} showed that there were intermolecular and intramolecular hydrogen bonds, featuring a hydroxyl stretching vibration. The absorptive peak at 2927.71 cm^{-1} was the C—H stretching vibration characteristic peak. The absorptive peak at 1730.78 cm^{-1} showed that the polysaccharide contained uronic acid, which was consistent with the results for the determination of the uronic acid content. The absorptive peak at 890.91 cm^{-1} ascribed to β-glycosidic linkages. β-D-grapes pyranose and β-D-galactose had absorptive peaks at 905–876 cm^{-1} and 876–886 cm^{-1}, respectively. The absorptive peak at 890.91 cm^{-1} needs to be further determined by other methods. The absorptive peak at 1395–1440 cm^{-1} was related to the C—O stretching vibration peak. The spectra also showed O—H variable angle vibration stretching at 1023.11 cm^{-1}, C—O stretching vibration at 1411.35 cm^{-1}, and C=O asymmetric stretching vibration at 1616.69 cm^{-1}.

3.4. Determination of monosaccharide composition

As shown in Fig. 2f and g, the GC–MS spectra profile of the purified Chinese yam polysaccharide presented two peaks with the retention time of 31.234 min and 31.692 min. It was concluded that the purified Chinese yam polysaccharide was composed of glucose and galactose with a molar ratio of 1.52:1 based on the total ion chromatogram of mixed standard monosaccharides. The methylation analysis of Chinese yam polysaccharide exhibited the presence

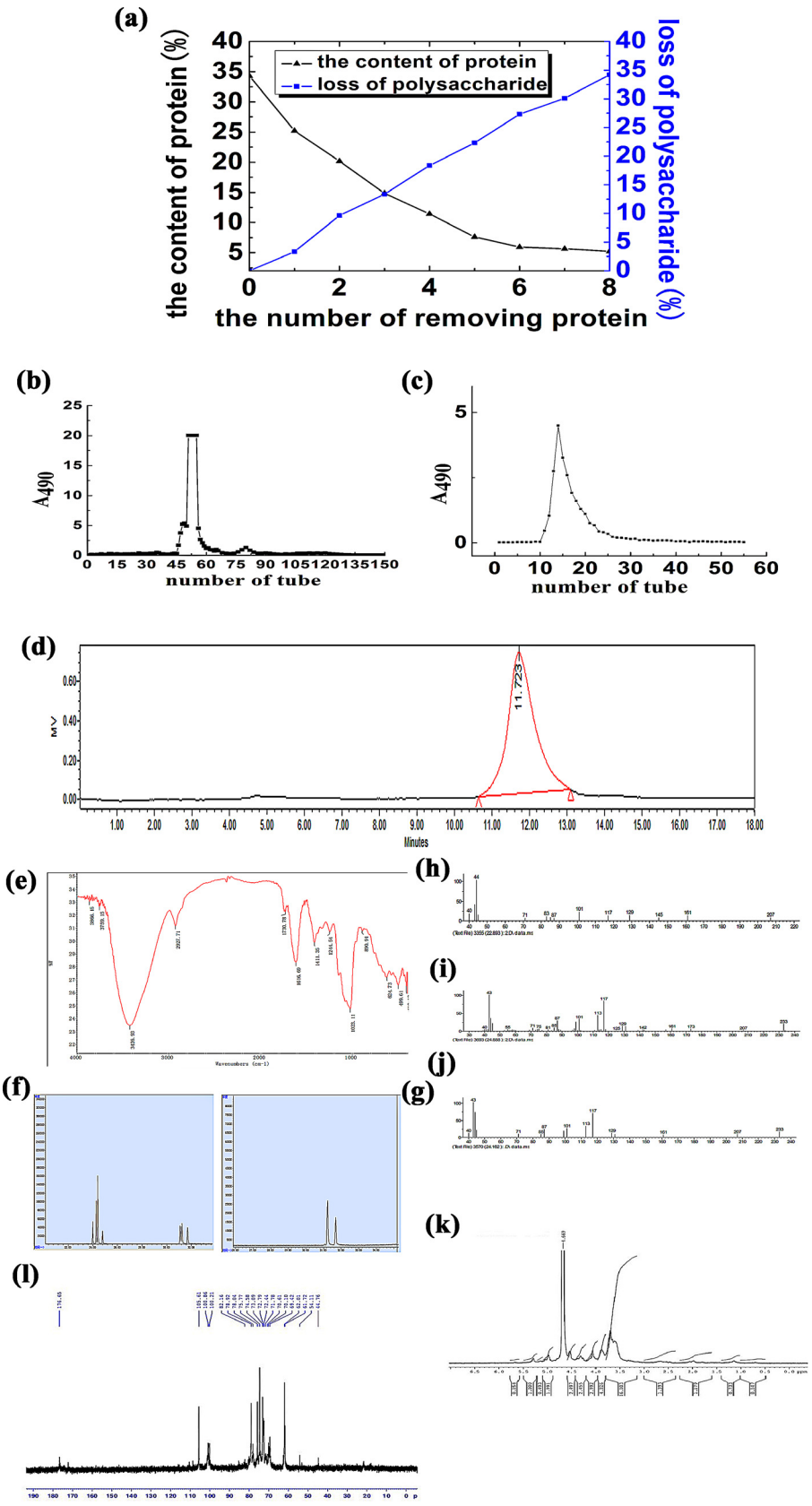

Fig. 2. (a) Variation of the Chinese yam polysaccharide yield and the protein content during deproteinization; (b) elution curve of the yam polysaccharide purified by HSCCC; (c) elution curve of Chinese yam polysaccharide by SephadexG-100; (d) HPLC–GPC analysis of Chinese yam polysaccharide; (e) FTIR spectrum of Chinese yam polysaccharide; (f) ion chromatography of the standard monosaccharide complex; (g) GC–MS spectrum of monosaccharide composition of Chinese yam polysaccharide; (h–j) mass spectrum of partially methylated Chinese yam polysaccharide; (k) ^{1}H NMR spectrum of Chinese yam polysaccharide; (l) ^{13}C NMR spectrum of Chinese yam polysaccharide.

Table 1
Results of the methylation analysis of Chinese yam polysaccharide.

Methylated sugar	Linkage type	Molar ratio	MS (*m*/*z*)
2,3,4,6-Me_4-Gal*p*	1→	1	44, 71, 87, 101, 117, 129, 145, 161
2,3,4-Me_3-Gal*p*	1,6→	1.22	43, 71, 75, 85, 87, 101, 113, 117, 129, 161, 173, 233
2,4,6-Me_3-Glc*p*	1,3→	3.13	43, 85, 87, 101, 113, 117, 129, 161, 207, 233

Table 2
Inhibition of Chinese yam polysaccharide on selected microorganisms.

Strains	Zone of inhibition (mm)					
	Control	5 (mg/mL)	10 (mg/mL)	15 (mg/mL)	20 (mg/mL)	25 (mg/mL)
Bacillus subtilis	—	—	—	—	—	—
Staphylococcus aureus	—	—	—	—	—	—
Nissl bacillus	—	—	—	—	—	—
Escherichia coli	—	11.06	11.25	11.69	11.87	11.29

"—" Means no zone of inhibition.

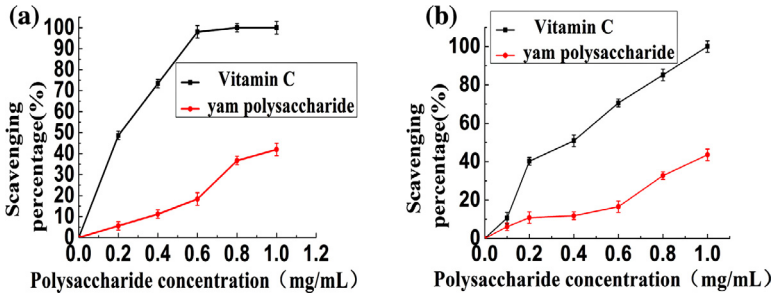

Fig. 3. (a) Scavenging percentage of Chinese yam polysaccharide to hydroxyl radical; (b) scavenging percentage of Chinese yam polysaccharide to superoxide radical. Vitamin C is used as the positive control in the free radicals scavenging experiments.

of three components (Fig. 2h–j). According to the peak areas, three types of residues were in the ratio of 1:1.22:3.13 (Table 1). Therefore, the galactose residues had two types of residues. On the basis of the methylation and GC–MS analysis, the purified polysaccharide mainly included 1,3-linked-glc, 1-linked-gal and 1,6-linked-gal glycosidic bonds.

3.5. NMR analysis

As shown in Fig. 2k and l, ^{1}H NMR and ^{13}C NMR spectra of the purified Chinese yam polysaccharide contained numerous peaks. In the anomeric region of the ^{1}H NMR spectrum at δ4.55 ppm, δ4.99 ppm, δ5.30 ppm showed three characteristic signals of two α-type glycosidic linkages and one β-type glycosidic linkage. In the anomeric carbon region of the ^{13}C NMR spectrum at δ105.61 ppm, δ100.86 ppm, δ100.21 ppm confirmed this conclusion, which was consistent with the ^{1}H NMR spectrum. The signal less than δ102 ppm corresponded to α-D-glucan, and the signal greater than δ102 ppm corresponded to β-D-glucan. Thus, it was reasonable to conclude that the 1,3-link glucose residues were β-configuration, and the other galactose residues were α-configuration. It was speculated that β-1,3-glucose, α-1-galactose, α-1,6-galactose might exist in the purified polysaccharide combined with methylation data. The signal at δ176.65 ppm corresponded to carboxyl resonance signal, which was consistent with FTIR data.

3.6. Antioxidative activity and antibacterial activity

As shown in Fig. 3a, the results showed that the same tendency was found for the scavenging capacity of free radicals between vitamin C and Chinese yam polysaccharide. At the experimental concentrations, Chinese yam polysaccharide showed an ability to scavenge hydroxyl radical, and the scavenging ability was improved with the increase in the polysaccharide concentration, though this ability was lower than that of vitamin C. Vitamin C exhibited a strong scavenging ability to hydroxyl radical, and the scavenging percentage was 100% at 0.6 mg/mL. At the experimental concentrations, Chinese yam polysaccharide and vitamin C exhibited a certain ability to remove superoxide anion, and the scavenging rate was improved with the increase in their concentrations. As shown in Fig. 3b, the scavenging rate of superoxide anion for vitamin C was increased rapidly. The scavenging percentage was 100% at 1.0 mg/mL of vitamin C, but the scavenging percentage of Chinese yam polysaccharide was only 43% at this concentration.

The antibacterial activity at the different polysaccharide concentrations was summarized in Table 2. The purified polysaccharide displayed a certain inhibitory activity against *E. coli*, but not against *B. subtilis*, *Staphylococcus aureus*, and *Nissl bacillus*. After Chinese yam polysaccharide was incubated at the concentrations from 5 to 25 mg/mL, the inhibition of experimental microorganisms were observed and compared. The inhibitory activity of Chinese yam polysaccharide against *E. coli* was improved with the increase in the sample concentration, with a minimal inhibitory concentration (MIC) of 2.5 mg/mL by the double dilution method. The antibacterial circle diameter at 25 mg/mL was smaller than that at 20 mg/mL. It was speculated that the excessive polysaccharide did not dissolve completely, so it was too viscous to penetrate outside oxford cups, which was unbeneficial for the inhibition of microorganisms.

4. Conclusion

The purification and structural characterization of Chinese yam polysaccharide and its activities were investigated in this study. The optimal extraction parameters were obtained by single-factor experiments, and were found to be as follows: pH 9.0, extract time 1.5 h, temperature 80 °C, and the ratio of water to Chinese

yam powder 1:8. Under the above conditions, the yield of Chinese yam polysaccharide was 5.19%. After the hot water extraction, the purification of Chinese yam polysaccharide was carried out by sevage deproteinization with sequential high-speed countercurrent chromatography and Sephadex G-100 size-exclusion column chromatography. The pure Chinese yam polysaccharide was obtained, and was detected by high performance liquid gel permeation chromatography. It was a single component with a molecular weight of 16,619 Da. Based on the analysis of the methylation, GC–MS, fourier infrared transformation, and ^{1}H NMR and ^{13}C NMR spectra, it was speculated that β-1,3-glucose, α-1-galactose, α-1,6-galactose might exist in the purified polysaccharide. The antioxidative activity showed that the purified polysaccharide could scavenge hydroxyl radical and superoxide radical. Moreover, it displayed a certain inhibitory activity against *E. coli*, with a minimal inhibitory concentration (MIC) of 2.5 mg/mL. This study provides a new insight into the future development and use of Chinese yam polysaccharide.

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