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Fractionation, preliminary structural characterization and bioactivities of polysaccharides from *Sargassum pallidum*

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ABSTRACT

Sargassum pallidum polysaccharides were fractioned using a DEAE-Sepharose fast-flow column and four polysaccharide fractions (SP-P1, SP-P2, PV-P3 and SP-P4) were obtained. Structural analyses indicated that SP-P2 and SP-P4 had higher molecular weights than SP-P1 and SP-P3. SP-P2, SP-P3 and SP-P4 comprised of fucose, rhamnose, arabinose, galactose, glucose, xylose, and mannose in a similar molar ratio, while SP-P1 did not contain arabinose. SP-P2 and SP-P4 had a similar number of (1 → 6) or (1→) glycosidic linkages (1 → 2) or (1 → 4) glycosidic linkages and (1 → 3) glycosidic linkages, while SP-P1 and SP-P3 contained a small number of (1 → 3) glycosidic linkages. SP-P2 exhibited better bioactivities than SP-P1, SP-P3 and SP-P4, including antioxidant, anti-hemolysis inhibitory, α-amylase and α-glucosidase inhibitory and antiproliferative activities. These data suggest that *S. pallidum* has four polysaccharide fractions with different structural features and bioactivities and SP-P2 has potential to be explored as a functional food or complementary medicine.

1. Introduction

Sargassum pallidum (Turn.) C. Ag., belonging to the family of Sargassum, is a kind of brown algae widely distributed in Yellow Sea and East China Sea. *S. pallidum* is rich in many nutritional components, such as vitamins, amino acids, dihomogammalinolenic acid, trace element, dietary fiber and polysaccharides (Ye et al., 2013). So far, this plant has been widely used as a Chinese health food in oriental countries (Luo et al., 2016). In China, this plant has also been recorded on "China's Traditional Medicine Dictionary" for its beneficial function of softening turbid-phlegm and blood-stasis hardness, eliminating phlegm, clearing interior heat and etc. It can regulate the balance of yin and yang in the body. Modern pharmacological studies demonstrated that *S. pallidum* polysaccharides possessed wide-ranging beneficial health-promoting properties, such as antioxidant, anticancer, hypolipidemic, and immunomodulatory activities (Li et al., 2012; Ye, Wang, Zou, Liu, & Zeng, 2008; Zhang, Luo, Bi, & Zhou, 2012).

Polysaccharides are considered as one kind of important biological response modifiers for good therapeutic and health-promoting effects. Moreover, due to no toxic and side effects, many bioactive polysaccharides have been widely studying and applying to the biochemical and medical fields (Huang, Wang, Zhou, Yang, & Wang, 2015; Yang & Zhang, 2009). Numerous studies indicated that the biological activities of polysaccharides closely depended upon chemical composition and structural characteristics, such as molecular weight, monosaccharide composition, glycosidic bonds, chain conformation and etc. (Chen, You, Abbasi, Fu, & Liu, 2015; Yang & Zhang, 2009). However, little work has been reported on the purification, chemical composition, structural feature, bioactivity, and structure-activity relationship of polysaccharides in *S. pallidum*.

Therefore, in order to further exploit and utilize *S. pallidum* polysaccharides in the functional food and pharmaceutical fields, *S. pallidum* polysaccharides (SPP) were extracted and then fractioned using a DEAE-Sepharose fast-flow column to obtain four polysaccharide fractions (named SP-P1, SP-P2, PV-P3 and SP-P4). The chemical composition, preliminary structural features and bioactivities of four polysaccharide fractions were comparatively investigated. The results obtained from this study will provide a scientific foundation for developing *S. pallidum* polysaccharide-based functional foods and its applications.

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2. Materials and methods

2.1. Material and chemicals

Sargassum pallidum was collected from the Yellow Sea (Shangdong, China) in September 2013 and identified by Dr. Chao-Hua Huang from the Ocean University of China. A voucher specimen was deposited with the School of Food Science and Engineering, South China University of Technology. Ascorbic acid, acarbose, bovine serum albumin (BSA), dextran standards, α-amylase, α-glucosidase, 2,2-azobis(2-methylpropionamidine)-dihydrochloride (AAPH), fluorescein sodium salt, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Standards of monosaccharide and glucuronic acid were purchased from Aladdin Chemistry Company (Shanghai, China). Dulbecco's modified eagle medium (DMEM) and penicillin-streptomycin were purchased from Gibco Life Technologies (Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from Zhejiang Tianhang Biological Technology Co. (Zhejiang, China). Human hepatocellular carcinoma (HepG2) cell line was purchase from Medical College of Sun Yat-Sen University. All other chemicals and solvents were of reagent grade.

2.2. Preparation of crude polysaccharides

The crude polysaccharides were extracted according to the method (Xu, Yan, & Zhang, 2012) with some modifications. Briefly, the dried *Sargassum pallidum* powder was refluxed twice with 95% (v/v) ethanol for 4 h at 70 °C to remove ethanol-soluble constituents. After filtration, the residue was collected and dried at 50 °C for 24–48 h. The dried residue was precisely weighed and extracted with boiling water for 2 h at a ratio of 1:20 g/mL. After two rounds of extraction, the supernatants were combined and concentrated to 1/4–1/5 volume under reduced pressure at 55 °C using a rotary evaporator (RE-52A, Yarong Co. Ltd., Shanghai, P. R. China). The proteins were removed using the Sevag reagent (chloroform:*n*-butyl alcohol = 4:1, v/v). Then, the extracts were mixed with macroporous resin AB-8 at a ratio of resin:solution 1:25 (v/v) and shaken for 12 h at room temperature. The solution was isolated by filtration and adjusted to a concentration of 80% (v/v) ethanol and kept overnight at 4 °C. The precipitates were separated on a centrifuge. The precipitates were then freeze-dried at −50 °C to obtain crude polysaccharides (SPP).

2.3. Separation and purification of SPP

The crude SPP solution (5 mL, 5 mg/mL) was applied to the DEAE-Sepharose fast-flow column (2.6 cm × 30 cm), and sequentially eluted with 200 mL of distilled water, followed by 0.1, 0.2, 0.3, 0.4, and 0.5 M NaCl solution at a flow rate of 1 mL/min. Fractions of 5 mL eluents were collected using an automatic collector (BSZ-100, Shanghai Precision Scientific Instrument Co., Ltd., P. R. China) and detected using the phenol-sulfuric acid method. The elution curve reflected the absorbance at 490 nm for each test tube. Water, 0.1 M, 0.2 M, and 0.3 M NaCl eluents (SP-P1, SP-P2, SP-P3 and SP-P4) were obtained (Fig. 1). Four fractions were concentrated and dialyzed using dialysis tubes (molecular weight cutoff = 3000 Da, Mym Biological Technology Company Limited, USA) in distilled water for 48 h followed by lyophilization.

2.4. Chemical composition determination

Total carbohydrate contents in samples were determined by the phenol-sulfuric acid colorimetric method using D-glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein contents were determined by the method of Lowry with BSA as the standard (Lowry, Rosebrough, Farr, & Randall, 1951). The sulfate contents were determined by the barium chloride-gelatin nephelometry method with some modifications (Zhang et al., 2015).

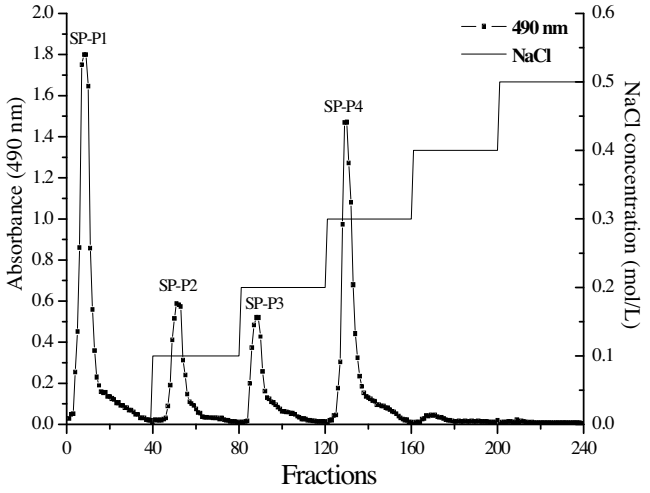

Fig. 1. DEAE-Sepharose fast-flow chromatogram of the SPP.

2.5. Homogeneity and molecular weight determination

The molecular weight distribution of each polysaccharide fraction was measured by a high-performance gel permeation chromatography (HPGPC) method as described previously (Li, Fu, Huang, Luo, & You, 2015). The process was performed on an Agilent 1260 instrument (Agilent, USA) equipped with TSK-G5000 PW_{XL} (7.8 × 300 mm i.d., 10 μm) and TSK-G3000PW_{XL} (7.8 × 300 mm i.d., 5 μm) in series. The sample solution (2 mg/mL) was passed through 0.22 μm filter, applied to a HPGPC column maintained at a temperature of 35 °C, and eluted with 0.02 M KH_2PO_4 (pH 6.0) at a flow rate of 0.6 mL/min. The 0.02 M KH_2PO_4 (pH 6.0) was chosen as the mobile phase due to its good separation efficiency for TSK gel column according to the manufacturer's instruction. The injection volume was 20 μL. Dextrans with different Mw (5.9, 9.6, 21.1, 47.1, 107, 200, 344 and 708 kDa) were used as standards. The calibration curves were obtained by plotting the elution volumes against the logarithm of their respective Mw. The regression curve equation obtained was $\mathrm{LogMw} = 36.81 - 5.55\,V + 0.33\,V^2 - 0.007\,V^3$ with a correlation coefficient of 0.9999.

2.6. Monosaccharide composition analysis

The samples (2 mg) were hydrolyzed with 4 mL of 2 M trifluoroacetic acid (TFA) at 110 °C for 6 h in a sealed tube. Excess TFA was removed by evaporation under reduced pressure at 45 °C. The residue was dissolved in 4 mL methanol and evaporated to dryness by a nitrogen blowing instrument at 50 °C. The residue was re-dissolved in 4 mL of distilled water, filtered by a 0.22 μm filter and then analyzed by a Dionex ICS 3000 (Sunnyvale, CA) with a CarboPac PA1 analytic column (250 × 2 mm) and a CarboPac PA1 guard column (50 × 2 mm). Fucose, D-glucose, rhamnose, xylose, mannose, galactose, arabinose, glucuronic acid and galacturonic acid were used as the standards. The monosaccharide content was calculated according to the linear regression equation of different concentrations of respective monosaccharide being proportional to the peak area.

2.7. Infrared (IR) spectrometry analysis

The IR spectra of SP-P1, SP-P1, SP-P3, and SP-P4 were determined using a Vector 33 FT-IR spectrophotometer (Bruker,