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Separation, purification and preliminary characterization of sulfated polysaccharides from *Sargassum plagiophyllum* and its *in vitro* anticancer and antioxidant activity

Veeraperumal Suresh[a], Namasivayam Senthilkumar[b,e], Ramar Thangam[c,d], Mayan Rajkumar[c], Chinnathambi Anbazhagan[a,*], Ramasamy Rengasamy[b], Palani Gunasekaran[c], Soundarapandian Kannan[d], Perumal Palani[b,*]

[a] *Department of Botany, Annamalai University, Annamalai Nagar, TN, India*
[b] *Centre for Advanced Studies in Botany, University of Madras, Chennai, TN, India*
[c] *Department of Virology, King Institute of Preventive Medicine & Research, Chennai, TN, India*
[d] *Proteomics & Molecular Cell Physiology Lab, Department of Zoology, Bharathiar University, Coimbatore, TN, India*
[e] *Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, TN, India*

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ABSTRACT

Sulfated polysaccharides (SPs) were identified in different portions of the thallus of *Sargassum plagiophyllum* C. Agardh, with TBO staining. SPs were extracted using a blade and purified by Q sepharose fast flow anion-exchange chromatography, resulting in SP fractions F1, F2 and F3, with molecular weights of 30, 35 and 20 kDa, respectively. An SP yield of 43.1% was obtained in F3, while F2 yielded a sulfate content of 21.9%. Furthermore, the *in vitro* anticancer and antioxidant activities of the polysaccharide fractions were evaluated. The F2 fraction showed higher anticancer activity against HepG2 and A549 cells than the other two fractions, with IC_{50} values of 600 μg/mL and 700 μg/mL, respectively. The normal breast epithelial cell line (HBL-100) exhibited IC_{50} concentrations of 1200 and 1400 μg/mL for crude sulfated polysaccharides (CSPs) and all SP fractions (F1–F3). These results indicated that the anticancer activity of F2 could be related to its sulfate content. However, the antioxidant activities of F1–F3 were low at their tested concentrations.

1. Introduction

There has been an increasing interest in seaweeds, mainly due to their bioactive components, which show great potential as anti-inflammatory, antimicrobial, antiviral and antitumoral treatments. Indeed, several species of algae have been found to be sources of polysaccharides and glycoproteins with immunostimulating, anti-tumoral and/or antiviral activity [1]. Among these, brown algae contain large amounts of cell-wall polysaccharides, most of which are sulfated polysaccharides and fucoidans [2]. The biological activities of algal polysaccharides have attracted the attention of phycologists. Recently, several investigations have been conducted on the isolation and characterization of polysaccharides derived from different *Sargassum* species, revealing the multiple biological activities of these species, including hepatic and renal protective, antioxidant, antitumor, anti-angiogenic, anti-inflammatory, anti-coagulant, anti-viral and anti-vasculogenic activities [3–10,41]. Generally, the biological activity of polysaccharides from marine algae is related to their molecular sizes, types of sugar, sulfate contents, types of linkage and molecular geometries [9]. Edible seaweeds are considered good sources of vitamins, minerals and non-caloric dietary fibers, and they are considered potential sources of biologically active ingredients [11]. *Sargassum plagiophyllum* C. Agardh is one of the industrially important brown algae occurring in the Rameshwaram coast off Tamil Nadu, India. Thus far, there has been no report on the biological properties of sulfated polysaccharides from brown seaweeds of the Gulf of Mannar.

Cancer is one of the most common diseases that threatens human life. Unfortunately, the drugs used for cancer therapy are toxic and affect not only cancer cells but also normal cells. Thus, finding novel, effective and nontoxic compounds from natural sources is important now more than ever before. The presence of anticancer activity could elevate the value of seaweed (*S. plagiophyllum*) as a food additive. In the present study, water soluble SPs were extracted, purified and investigated for their anticancer

* Corresponding authors. Tel.: +91 9597007235; fax: +91 4144 238080.
E-mail addresses: veeraperumalsuresh@gmail.com (V. Suresh), senthilkumarin@yahoo.co.in (N. Senthilkumar), thangam1985@yahoo.co.in (R. Thangam), drcanbu@gmail.com (C. Anbazhagan), palanii7@yahoo.com (P. Palani).

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potential in human liver cancer (HepG2) and lung cancer (A549) cell lines.

2. Materials and methods

2.1. Collection of S. plagiophyllum

S. plagiophyllum (fresh weight) was collected along the coast off Mandapam (Lat. 09°17′N; Long. 79°08′E), Palk Bay, Tamil Nadu, India, in November 2009. After thorough washing with seawater and manual sorting to remove epiphytes, the fresh biomass was exhaustively washed with tap water followed by distilled water, then shade dried and ground to pieces of diameter of approximately 1 mm.

2.2. Identification of SPs (O'Brien et al. [12])

The identification of SPs was carried out using the Toluodine Blue-O method, in which 0.05% Toluodine Blue-O (TBO) was prepared by dissolving 0.05 g of TBO in benzoate buffer at pH 4.4. Free hand-thin sections of live samples were stained with TBO [12].

2.3. Extraction of sulfated polysaccharides

A total of 100 g of algal powder of the entire thallus and different sections of the thallus (dry biomass) was soaked separately in an acetone–methanol solvent system (7:3) for two days in a shaker at 200 rpm (Remi, Mumbai). The process was repeated twice to ensure the complete decoloration and defatting of dry biomass. This biomass was dried into a powder and dispersed in 1 L of 0.1 M HCl for 24 h with constant stirring at room temperature. The pellet was re-extracted as above, and the supernatants were pooled. The resulting supernatant was kept at 4 °C overnight and precipitated with two volumes of absolute ethanol 1:1 (v:v). The precipitate was collected, dissolved in water and dialyzed against water using a membrane (MW CO 14,000, Hi Media, Mumbai, India) at 4 °C for two days; then, the dialysate was freeze-dried. Maximum sulfate containing SPs from parts of the thallus was subjected to further processing.

2.4. Purification of SPs by anion-exchange chromatography

Crude polysaccharides dissolved in 0.1 M sodium phosphate buffer (pH 7.2) were applied to a column of Q sepharose fast flow (4 cm × 25 cm), followed by step-wise elution with 0.1 M sodium phosphate buffer and then solutions of 0.2, 0.7 and 1.5 M sodium chloride at a flow rate of 60 mL/h. Eluant (5 mL/tube) was collected, and the carbohydrate content was determined using a phenol–sulfuric acid method, using fucose as the standard [13]. Finally, three fractions of polysaccharides were obtained, dialyzed with water and lyophilized for further study.

2.5. Chemical analysis

The total sugar content was determined using the phenol–sulfuric acid method [13]. Sulfate content was also measured [14]. Total uronic acid content was analyzed colorimetrically by an m-phenyl phenol method using glucuronic acid as the standard [15,16].

2.6. Monosaccharide identification

To determine the neutral sugar composition of the extracted sulfated polysaccharides, the samples (5 mg each) were added to trifluoroacetic acid (100% TFA, 4 mL) in a round-bottom flask. The mixtures were left overnight at ambient temperature and subsequently refluxed for 2 h. The solutions were then diluted to 80% TFA using deionized water. After refluxing for 30 min, the solutions were diluted again to 30% TFA using deionized water and refluxed for 4 h. The TFA was removed using a rotary vacuum evaporator. Deionized water was then added to the solids to wash them, and then the water re-evaporated. This procedure was repeated several times until the hydrolysates obtained became neutral. The dry hydrolysate solids were finally dissolved in deionized water (5 mL) and analyzed by Agilante 1100 High Performance Liquid Chromatography (HPLC) (Agilante Technologies, Santa Clara, CA, USA) on a C18 column (ZORBAX Eclipse XDB-C18, 4.6 mm × 150 mm 3.5 μm).

2.7. Agarose gel electrophoresis

The purity of SPs was checked with agarose gel electrophoresis according to the method described by Björnsson [17]. SPs (1 mg/mL, dry weight) were electrophoresed in 1.0% agarose gels using a 0.01 M tris/acetate running buffer (pH 8.3). The gel was run at 90 V for 90 min and stained with 0.02% (w/v) toluidine blue O in a 3% acetic acid solution containing 0.5% (v/v) Triton X-100. The gels were destained in 3% acetic acid (Fig. 1b).

2.8. Molecular weight analysis

The molecular mass of fucoidan was analyzed by gel filtration chromatography. The purified SPs (10 mg) were chromatographed on a Sepharose 6B column (90 cm × 1.0 cm) using a 100 mM sodium phosphate buffer (pH 7.2) as the eluant. The flow rate of the column was 0.6 mL/min., and fractions of 2 mL were collected and checked with a phenol–sulfuric acid reaction [13]. The column was calibrated with standard dextrans (500, 70, 40 and 10 kDa).

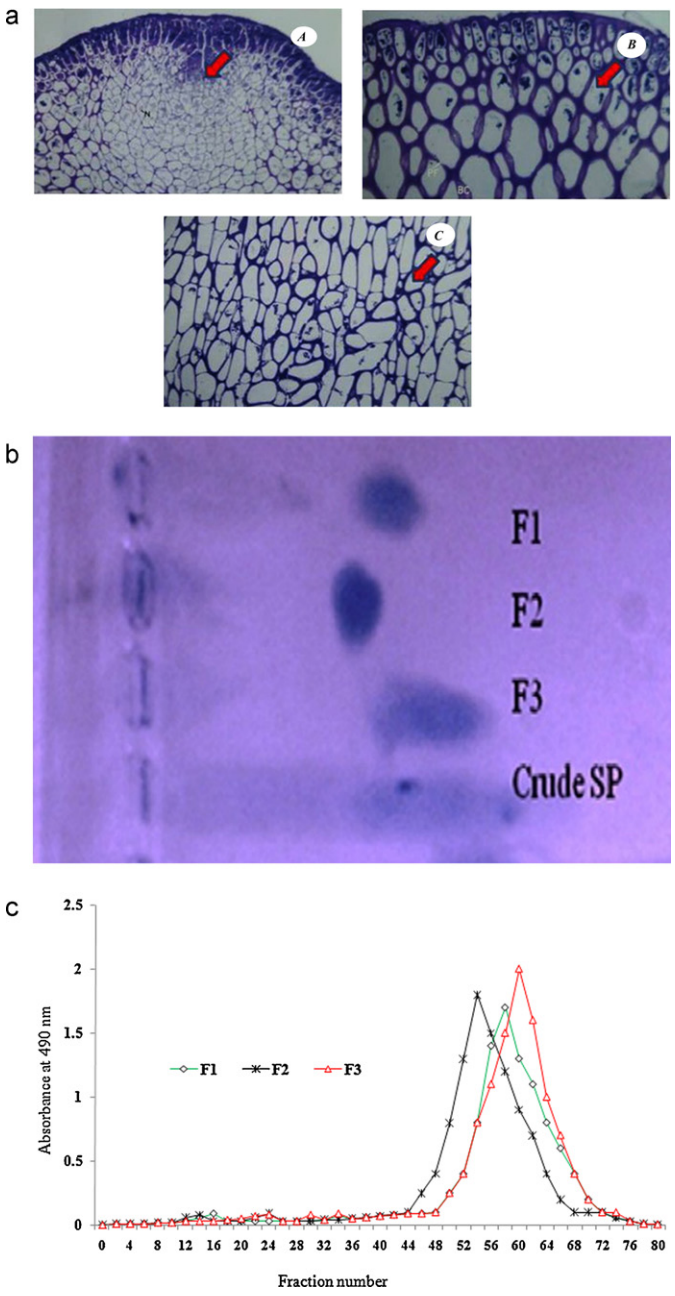

Fig. 1. (a) A. Longitudinal section of the leaf region, 300×. B. Cross section of the stem region, 625×. C. Cross section of the holdfast region, 625×. (b). Agarose gel electrophoresis of different fractions. (c) Gel filtration profile of different fractions from Sepharose 6B column chromatography.

2.9. Cell culture and maintenance

The selected cell lines, HepG2, A549 and HBL-100, were purchased from the National Center for Cell Sciences (NCCS), Pune, India. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 2 mM L-glutamine and balanced salt solution (BSS) and adjusted to contain 1.5 g/L Na_2CO_3, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100 μg) were added at 1 mL per liter. The cells were maintained at 37 °C with 5% CO_2 in a humidified CO_2 incubator.