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## Isolation, Characterization and Anticoagulant Activity of Sulfated Polysaccharides from Brown Algae *Sargasam wightii* Greville

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**Abstract:** The aim of the present study is to isolate the sulfated polysaccharides from marine macro algae *S. wightii*. The crude sulfated polysaccharides were fractionated by anionic resin (Amberlite IRA-900) and the separated active fractions were confirmed by agarose gel electrophoresis. The active fractions were pooled, dialyzed and purified by molecular sieve (Sephadex G-100) chromatography. The molecular weight of fractionated as well as purified sulfated polysaccharides was determined through gradient polyacrylamide gel electrophoresis (PAGE) and the disaccharide profile of purified sulfated polysaccharides was also analyzed. The structure of purified sulfated polysaccharides was dogged by <sup>1</sup>H-NMR spectrum. The elements such as Carbon, Hydrogen and Nitrogen (CHN) and Activated Partial Thromboplastin Time (APTT) were observed from the purified sulfated polysaccharides. The hexosamine, uronic acid and the CHN content in the purified sulfated polysaccharides were found to be low (except molecular weight) when compared to standard heparan sulfate. The molecular weight of purified sulfated polysaccharides have 6,500 Da and the concentration of disaccharide profile such as 5.52% of uronic acid, 3.47% hexosamine and 5.4% of sulfate. APTT of fractionated and purified sulfated polysaccharides was found to be 24.6 and 31.1 IU mg<sup>-1</sup>, respectively. These results suggest that the sulfated polysaccharides from *S. wightii* could be an alternative source of anticoagulant.

**Key words:** Sulfated polysaccharides, *S. wightii*, <sup>1</sup>H-NMR and APTT

### INTRODUCTION

The novel biological resources which are widely available in nature have been used as a source of therapy for thousands of years. To a large extent of the world's biological variety remains unexplored and now-a-days, the searches for new bioactive agents from natural sources are of much importance. Several of these unique compounds have shown pharmacological activities for many of the boring diseases like cancer, AIDS, diabetes, arthritis, etc. (Smit, 2004). The marine milieu is an outstanding basin of bioactive natural products has led to the innovation of many potentially active agents consider worthy of clinical applications (Saravanan *et al.*, 2009). Seaweeds have been used since ancient times as food, fodder and fertilizer and as natural sources with therapeutic and bioactive molecules for treating multiple diseases. Today seaweeds are the raw material for industrial production of agar, carrageenan and alginates, but they continue to be widely consumed as food in Asian countries (Mishra *et al.*, 1993).

Seaweed resources have been used for isolation of major metabolites such as polysaccharides, lipids, proteins, carotenoides, vitamins, sterols, enzymes, antibiotics and many other fine chemicals (Ibtissam *et al.*, 2009). Sulfated polysaccharides are one of the anticoagulant compounds present in marine algae and occur in the form of sulfated fructose and sulfated galactans (Painter, 1983). Some algal species trigger anticoagulant activity through protein or glycoprotein-like compounds (Yasuda *et al.*, 2004) which binds to the serine protease, an inhibitor of antithrombin, producing a complex which accelerates the protolysis of the enzyme responsible for coagulation. The preparation of heparin and sulfated glycosaminoglycans from natural sources is expensive and therefore an alternative is desirable (Cassaró and Dietrich, 1997). Hence, an attempt was made to isolate sulfated polysaccharides from *S. wightii* which could be a potent alternative for anticoagulant substance. The brown algae harvested from the earth's marine are beyond doubt one of the most expensive gifts of the great deep. *S. wightii* is one of the marine brown algal species

broadly found in India, with incredible biological applications and are known to be well-off in sulfated polysaccharides content. Sulfated polysaccharides were found to clutch large pharmacological applications.

## MATERIALS AND METHODS

**Sample collection:** Seaweeds were collected from Kannyakumari, Tamil Nadu, India, washed and dried at 50°C under ventilation in an oven and ground with blender.

**Fractionation of sulfated polysaccharides:** Fifty grams of the dried powder were suspended with 5 volumes of 0.1 M NaCl and the pH of the mixture was adjusted to 8.0 with NaOH. Ten milligram of protease (Sigma, St. Louis, USA) was then added to the mixture for proteolytic digestion. After 24 h incubation at 60°C with agitation and periodical adjustments of pH, the mixture was filtered through cheese cloth. To the filtrate 10 g of Amberlyte IRA-900 (chloride form, pH 7.0), ion-exchange resin (Sigma, St. Louis, USA) were added and the resulting mixture was agitated for 24 h at room temperature under a layer of toluene. The suspension was then filtered again through the same cheese cloth. The resin retained in the cloth was washed with 1 L of water at 50°C and subsequently washed with 1 L of 0.1 M NaCl at room temperature (Leite *et al.*, 1998).

**Purification of sulfated polysaccharides:** The active sulfated polysaccharides (confirmed by agarose electrophoresis) eluted from the ionic resin with NaCl were pooled and further purified by molecular sieving in Sephadex G-100(100×1.5 cm). About 50 mg of fractionated sulfated polysaccharides were applied in 2 mL of distilled water and the fractions (3 mL) were eluted from the column with 0.2 M acetic acid. Purified sulfated polysaccharides were dialyzed (molecular membrane cutoff >10 kDa) against distilled water and freeze-dried (Saravanan and Shanmugam, 2010).

**Agarose and gradient PAGE:** Agarose gel electrophoresis of fractionated sulfated polysaccharide in the discontinuous buffer barium acetate/1, 3-diaminopropane acetate was performed (Toida *et al.*, 1997). The average molecular masses of fractionated and purified sulfated polysaccharides were analyzed by gradient PAGE (Edens *et al.*, 1992).

**Determination of disaccharide profile:** Uronic acid, hexosamine and sulfate contents of purified sulfated

polysaccharides were estimated by following the methods of Bitter and Muir (1962), Wagner (1979) and Terho and Hartiala (1971), respectively.

**<sup>1</sup>H-NMR analysis:** <sup>1</sup>H-NMR of purified sulfated polysaccharides (Varian BRUKER-500 MHZ) was performed (Sudo *et al.*, 2001) and compared with standard heparan sulfate. The thoroughly dried sample was re-dissolved in 0.5 mL of 2 H<sub>2</sub>O (99.6%) and transferred to the NMR tube. All spectra were determined on a Varian BRUKER-500 MHZ spectrometer equipped with 5 mm triple resonance tunable probe with standard Varian software at 294.9 K on 700 µL samples at 0.5-1.0 mM. The HOD signal was suppressed by pre-saturation during 3 sec.

**Element analysis:** In the CHN operating mode the PE 2400 Series II CHNS/O Analyzer uses a combustion method to convert the sample is first oxidized in a pure oxygen environment using classical reagents. Products produced in the combustion zone include CO<sub>2</sub>, H<sub>2</sub>O and N<sub>2</sub>. Elemental, such as halogens and sulfur are removed by scrubbing reagents in the combustion zone. The resulting gases are homogenized gases are allowed to de-pressurize through a column where they are separated in a stepwise steady-state manner and detected as a function of their thermal conductivities.

**Blood coagulation assay:** APTT of fractionated and purified sulfated polysaccharides were performed using kit obtained from Lexington, USA.

## RESULTS

The yield of sulfated polysaccharides from *S. wghitii* was found to be 3.56 g kg<sup>-1</sup> in dried seaweeds. Among the collected fractions, only 3 were offered sulfated polysaccharides (1.0, 1.5 and 2.0 M NaCl). These active sulfated fractions have fast mobility and showed only single band in agarose gel electrophoresis which was compared with the standard heparan sulfate (Fig. 1). The molecular weight of fractionated sulfated polysaccharides was observed as 7,500 Da and purified sulfated polysaccharides has 6,500 Da which were compared with the standard (Fig. 2). The disaccharide profile of purified sulfated polysaccharides showed 4.35% of uronic acid, 2.78% of hexosamine and 2.8% of sulfate contents whereas the purified sulfated polysaccharides recorded 5.52% of uronic acid, 3.47% hexosamine and 5.4% of sulfate.

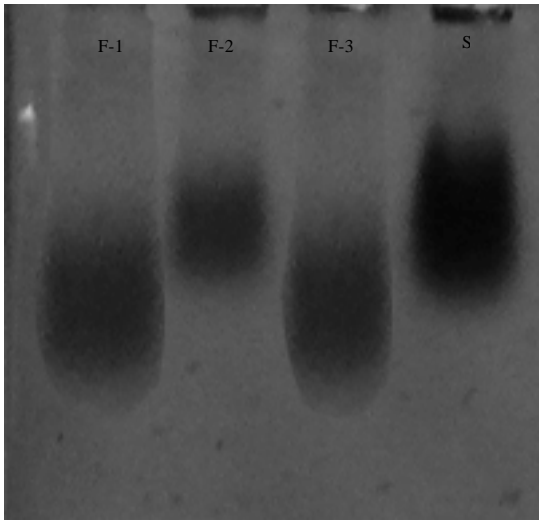


Fig. 1: Agarose gel electrophoresis of sulfated polysaccharides from *S. wightii*, F-1: 1.0 M NaCl, F-2: 1.5 M NaCl, F-3: 2.0 M NaCl and S: Standard

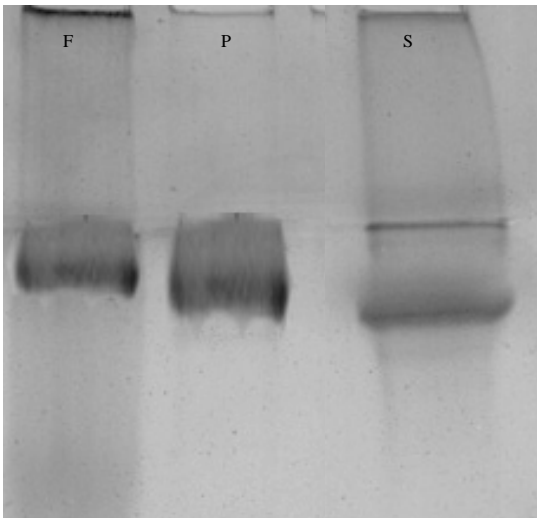


Fig. 2: Gradient PAGE of sulfated polysaccharides from *S. wightii* F: Fractionated sulfated polysaccharides, P: Purified sulfated polysaccharides and S: Standard

Furthermore, the <sup>1</sup>H-NMR (Fig. 3) showed presence of iduronic acid and N-acetyl glucosamine from of purified sulfated polysaccharides *S. wightii* with impurities of other hexoses which is compared with standard (Fig. 4). The elemental analysis such as carbon, hydrogen and nitrogen of sulfated polysaccharides from *S. wightii* were represented in Fig. 5. From this figure there is major

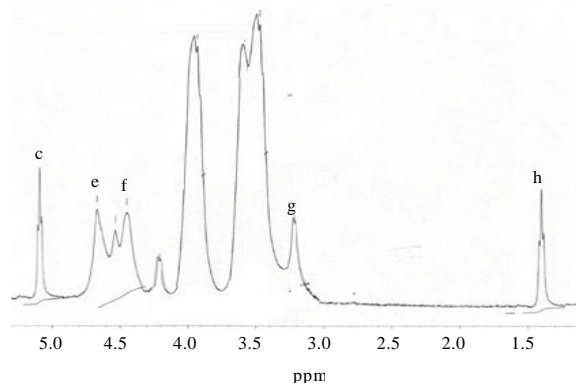


Fig. 3: <sup>1</sup>H-NMR sulfated polysaccharides spectrum purified of *S. wightii*

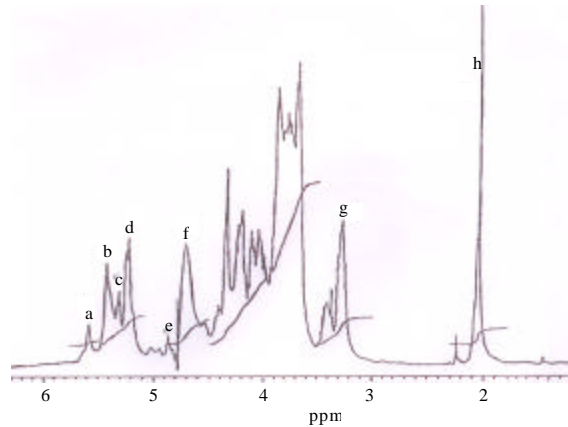


Fig. 4: <sup>1</sup>H-NMR of standard heparan sulfate labelled peaks are assigned as (a) H-1 of GlcNpS→GlcAp, (b) H-1 of GlcNpAc→IdoAp, (c) H-1 of IdoAp, (d) H-1 of GlcAp; (e) H-5 of IdoAp, (f) H-4 of IdoAp, (g) H-2 of GlcAp and (h) methyl of GlcNAc

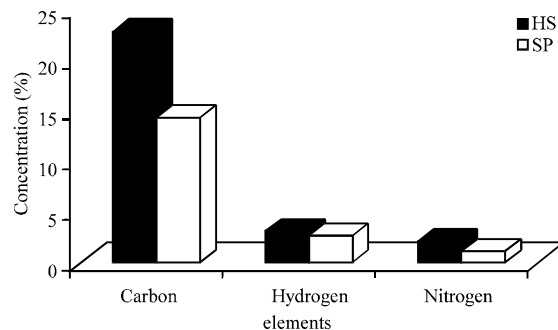


Fig. 5: CHN analysis of purified sulfated polysaccharides from *S. wightii*, HS: Heparan sulfate, SP: Purified sulfated polysaccharides of *S. wightii*

difference between the contents of carbon (22.80%), hydrogen (3.08%) and nitrogen (2.08%) found to be in HS

from bovine where as the marine brown algae recorded the carbon (14.33%), hydrogen (2.64%) and nitrogen (1.11%) correspondingly. Finally the APTT activities of fractionated and purified sulfated polysaccharides from *S. wightii* were found to be 24.6 and 31.1 IU mg<sup>-1</sup>, respectively.

## DISCUSSION

In the present investigation sulfated polysaccharides from *S. wightii* was estimated and the yield of sulfated polysaccharides from brown algae was low when compared to the result of Leite *et al.* (1998), who extracted total polysaccharide from Sulfated polysaccharides *Spatoglossum schroederi*. The molecular weight of purified sulfated polysaccharides extracted from *S. wightii* was about 7,500 Da. This is accordance with the result of Nardella *et al.* (1996), who reported the low molecular weight fucoidan (7.2 kDa) from brown seaweed *Ascophyllum nodosum* using ion-exchange chromatography.

The disaccharide profile of purified sulfated polysaccharides from *S. wightii* has recorded more when compared to fractionated sulfated polysaccharides from *S. wightii*. Larsen *et al.* (1966) and Medcalf and Larsen (1977) have reported a fucoidan from *A. nodosum* contained a central core of β-D-glucuronic acid with branches of fucose. In CHN content of purified sulfated polysaccharides was low as judged against to marine scallop heparan sulfate (Saravanan and Shanmugam, 2011). The variation of CHN contents may be due to the variation of disaccharide pattern present in the heparin complex isolated from different sources (Pugazhendi, 2002).

The APTT activity of *S. wightii* was found to be high when compared to the anticoagulant activity of sulfated xylogucoglyuronan from *S. schroederi* (Leite *et al.*, 1998). The increase in the anticoagulant activity of sulfated polysaccharides from *S. wightii* may be due to the high sulfate content, this was supported by the result of Mao *et al.* (2006) who stated that difference in the potency of thrombin inhibition may be due to the sugar residue, sulfate content and their structural features. The anticoagulant activity of sulfated polysaccharides was tested *in vitro* by APTT assay and the values were compared with those of standard heparan sulfate (~170 IU mg<sup>-1</sup>) (Saravanan and Shanmugam, 2011).

## CONCLUSION

The APTT activity of purified sulfated polysaccharides from *S. wightii* was low when compared

to standard. This result suggest that sulfated polysaccharide less effective *in vitro* and which can increased by purify the sulfated polysaccharides through HPLC and this could be used as an potential anticoagulant in future.

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