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Sulphated polysaccharides of brown algae and their uses

I. RAJENDRAN[1*], S. CHANDRASEKAR[1] AND P. VIJAYAGOPAL[2]

[1]*Mandapam Regional Centre, ICAR-CMFRI, Marine Fisheries - 623 520, Ramanathapuram District, Tamil Nadu*
**E-mail : cmfrirajendran@gmail.com*
[2]*Central Marine Fisheries Research Institute, Kochi - 682 014, Kerala*

ABSTRACT

In the present study works carried out on the isolation and spectral charaterization of fucoidan and AOS of algin obtained from the brown seaweed *Sargassum wightii* growing at Kilakarai, Tamil Nadu are presented and discussed.

Introduction

Seaweeds have been the staple food since ancient times (Dillehay *et al.*, 2008). The hydrocolloid or sulphated polysaccharides (SPS) as otherwise called are of high demand worldwide owing to their increased functional food nature not only for humans but plants and animals also (Bixler and Porse, 2011; Craigie, 2011; Løvstad Holdt and Kraan, 2011). SPS of marine algae are commercial important products and unique in their structure with respect to the presence of sulphate groups to bring out special properties unlike the plain conventional polysaccharides. They have wide applications in food as emulsifiers, stabilizers or thickeners apart from the recent investigations to develop them as nutraceuticals, cosmetics, pharmaceuticals and functional foods (Burtin 2003; Kim and Chojnacka, 2015). The major basic monomers comprising these SPS are galactose (agar and carrageenan), mannose, gulose (algin) and fucose (fucoidan) fig. 1a,b.

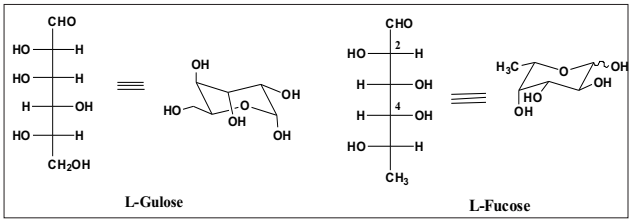

Fig. 1b

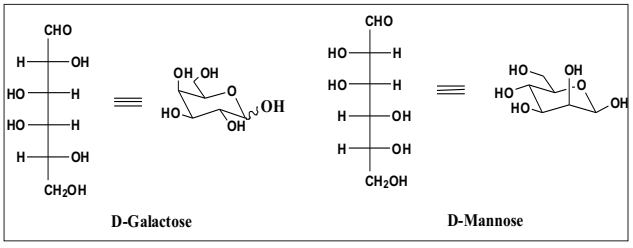

Fig. 1a

Commercially important major SPS are agar (Fig.5), algin (Fig.2) and carrageenan (Fig.6) obtained from red, brown and red algae respectively. Minor SPS are fucoidans (sulphated fucose, Fig.8), xylans, ulvans (green seaweeds), laminarin (β-1,3-glucan) and floridean starch (amylopectin like glucan). The constitution of each polymer depends on the degree of polymerization and sulphate content, species, season, location of the algae (Kim and Chojnacka, 2015). Agar is chemically 1,3-linked β-D-galactopyranose units attached to 1,4-linked 3,6-anhydro-α-L-galactopyranose residues. Carrageenan is sulphated 1,3- and 1,4-linked D-galactose with variation in number and position of sulphate groups (Fig.5 & 6). Algin is an anionic linear polymer composed of mainly by α-L-guluronic acid (G) and β-D-mannuronic acid (M) with sulphation either at C-2 and C-4 positions for 1→3 linkage or C-2 and C-3 positions for 1→4 linkage. This polymer is heterogenous in nature having composed of homopolymer containing G or M only and heteropolymeric with both M and G units (Hirst and Rees, 1965; Haug, *et al*., 1966) (Fig.2,3 & 4). Furcellaran and laminaran are other species specific phycocolloids.

SPS of brown seaweeds

The SPS derived from brown seaweeds are having versatile properties in the field of cosmetics, medicine and

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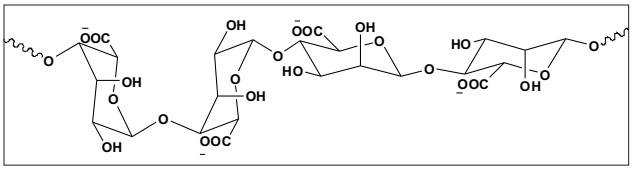

Fig. 2. *Structure of algin*

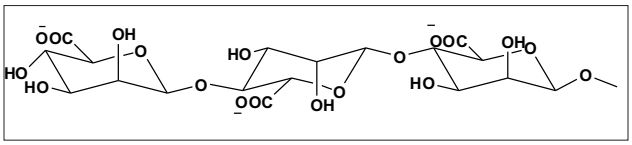

Fig. 3. *Mannuronate*

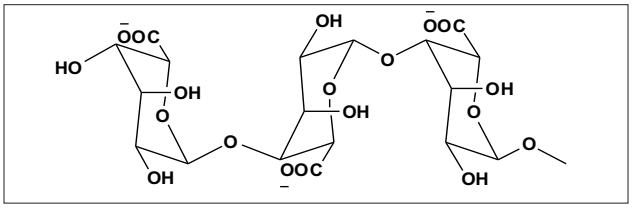

Fig. 4. *Guluronate*

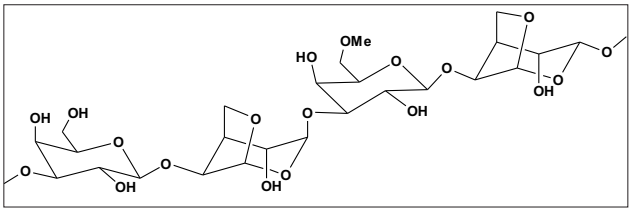

Fig. 5. *Agar*

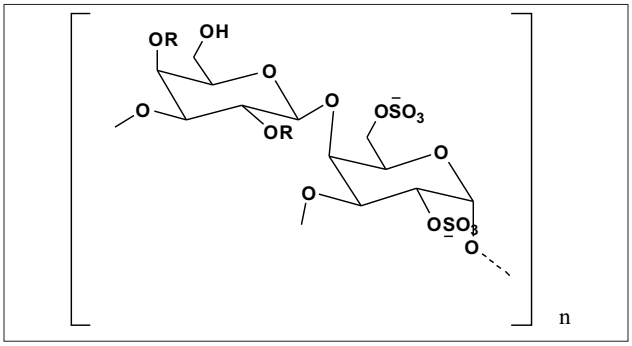

Fig. 6. *Carrageenan, R=H or SO_3*

nutraceuticals. Major seaweed sources are *Laminaria* sp., *Fucus* sp., *Sargassum* sp., *Ascophyllum* sp., *Undaria* sp., *Macrocystis* sp., *Ecklonia* sp., *Padina* sp. They have definite activity for which they are administered, eg. Mariculture (Bagni *et al*., 2005; Chotigeat *et al*., 2004). SPS with lower molecular weights of 50kDa were found to have higher antitumor activity (Ye *et al*., 2008). Algin is useful in wound healing compositions (Glyantsev, *et al*., 1993; Doyle, *et al*., 1996), control over cholesterol and blood glucose level for weight management (Torsdottir *et al*., 1991; Zee, 1991) and controlling of acid reflux

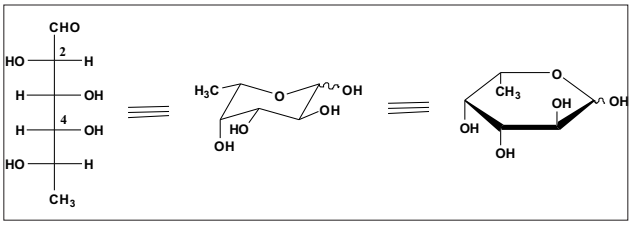

Fig. 7. *beta-L-Fucose*

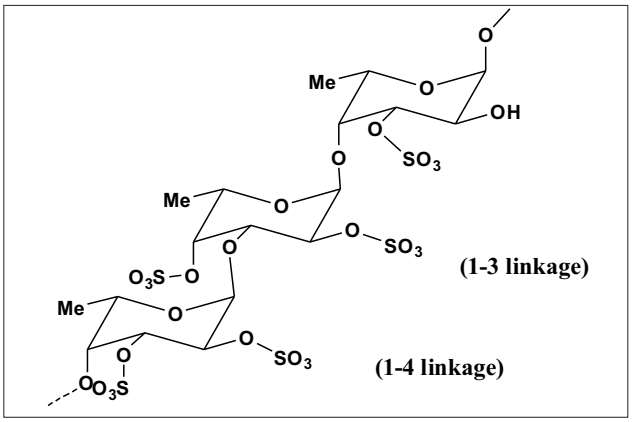

Fig. 8. *Sulphated fucose*

(Klinkenberg-Knol *et al*., 1995). Fucoidan is composed mainly of deoxy sugar units of β-L-fucose with sulphate substitution interlinked either by 1,3- or 1,4- linkages (Fig. 7 & 8). Its extensive applications and properties include anticoagulant (Chevolot *et al*., 1999), anti arteriosclerosis (Durig *et al*., 1997), antioxidant (Hu *et al*., 2001; Xue *et al*., 2004), anti cancer (Han *et al*., 2008), antitumor (Ellouali *et al*., 1993; Alekseyenko *et al*., 2007), anti HIV (Trinchero *et al*., 2009), antiviral (Lee *et al*., 2004), etc. Algin oligosaccharides (AOS) have been found to have antiviral properties (Meiyu *et al*., 2003; Queiroz *et al*., 2008), increased growth rate of *Nannochloropsis oculata* (Yokose *et al*., 2009), etc. AOS rich in mannuronate (M) or guluronate (G) unit can be obtained by various chemical (Haug, *et al*., 1966) and enzymatic methods.

Materials and Methods

Sargassum wightii was collected from Kilakarai coast of Gulf of Mannar. Fresh seaweeds were washed with seawater and transported to laboratory. It was then washed with tap water to remove remaining sand and debris. It was shade dried and coarsely powdered using a domestic mixer. ^{1}H NMR experiment was done in D_2O by Bruker-AV400MHz spectrometer at Sophisticated Instruments Facility, NMR Research Centre, Indian Institute of Science, Bangalore. FT-IR spectra were recorded on Nicolet™iS5 Thermo Scientific, USA with Omnic software in KBr pellets in 4000-400 cm^{-1} region at Alagappa University, Karaikudi. pH reading was done using Multi Parameter PTTestr™35 (OAKTON).

Isolation of fucoidan

Hot water extraction method

To the algal powder of *S. wightii* (2 kg), water (60 lit) was added and macerated while heating to 95°C for ½ an hour. The clear filtrate was decanted into five evaporating trays and kept for air evaporation. Recovery of fucoidan was attempted with the solvent mixture of ethanol-chloroform (87:13) with the addition to clear algal extract. The solution was kept overnight and the precipitate (ppt) was filtered through filter paper to get dark brown ppt. Further addition of alcohol to this solution gave brown ppt and the ppt was pooled. Yield < 1%.

Acidic extraction

Algal powder *S. wightii* (1 part) was treated with 0.2N H_2SO_4 (15 part) and kept overnight. The clear extract was concentrated by air evaporation on trays and the volume was reduced to half. The solution was neutralized in lots with sodium carbonate solution (15%) and NaCl was added to ease precipitation. Equal volume of alcohol was added to the concentrated extract to get dark brown ppt. The crude ppt was purified by dissolving in water, kept overnight and salty residue was removed. The clear aqueous solution was neutralized with sodium carbonate (1.5%) solution and $CaCl_2$ solution to remove algin present in the solution. Alcohol was added to this solution to the final concentration of 60% of alcohol to get light grey ppt of fucoidan. The ppt was dried and powdered to get overall yield of about 1.5%. The product was analyzed by ^{1}HNMR. The purity of the product was checked by HPLC (Fig.9).

Isolation of algin

Fucoidan free algal powder (1kg) was extracted with soda solution (Na_2CO_3, 1.75%) and the mass was diluted with water to filter further the extract. The combined extracts were treated with alcohol to get the sodium alginate precipitate which was then squeezed and washed well with alcohol. This squeezed alcoholic algin mass was used for degradation.

Degradation of algin

The alcoholic sodium alginate (200g) was heated in a beaker over a water bath to remove alcohol. Sulphuric acid (1N, 750ml) was added to the sodium alginate powder and kept for 7½ hrs with heating. The contents were filtered to get the filtrate and the residue. The residue was again extracted with another lot of H_2SO_4 (200ml) and the filtrates were combined. The acid degraded residue was extracted with water at pH levels of 2, 4, 6 and 8 using 300ml each of RO water using Na_2CO_3 solution. The products from each pH value of the extract were recovered using alcohol. The yield of products at various pH levels recovered was: pH 2 = 2%; pH 4 = 65%; pH 6 = 23%; pH 8 = 4.6%; Residue = 4.8%

Results and Discussion

Fucoidan

Among the various methods attempted for good recovery of fucoidan, the method involved acidic extraction was good and quantitative compared with hot water extraction. Since fucoidan is a heterogenic mixture with prominent α-L sulphated fucose as major units and sulphated galactose to a lesser extent, the spectral data pertaining to these hexose units were observed. The IR spectrum had broad peak at 1325 cm^{-1} corresponds to CH_3 of fucose; 1200 cm^{-1} to S=O stretching; 750-800 cm^{-1} to C-O-S stretching; 845 cm^{-1} to SO_4 in axial position, 3465 cm^{-1} to OH group (Fig. 10). These data are conforming to the reported values of 1→3 α-L-fucopyranosyl group (Yu *et al.,* 2014).

^{1}H NMR: The aqueous extract of *Sargassum* sp. afforded the spectrum with the integral regions of 1.0 - 3.0 δppm and 3.0 - 5.0 δppm (Craigie *et al.,* 2008; Grasdalen *et al.,* 1979). This region may be considered as fingerprint for the *Sargassum* sp. The peaks with δ values between 5.12 - 5.789 show the deshielded value of anomeric hydrogen, H-1 in α-position.

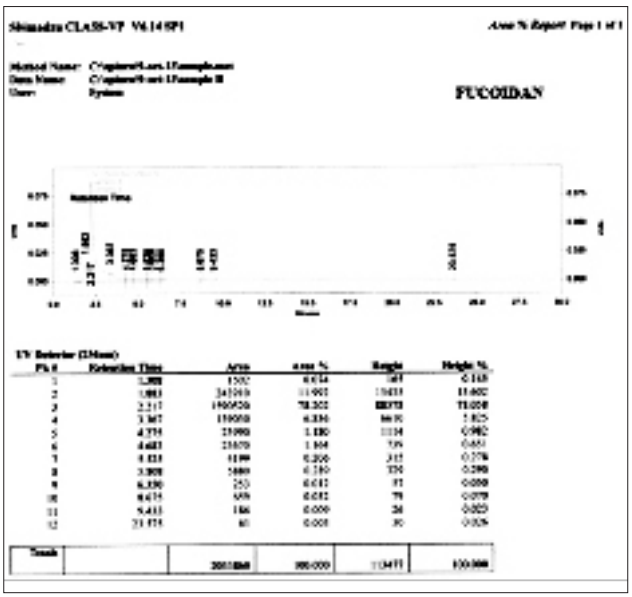

Fig. 9.

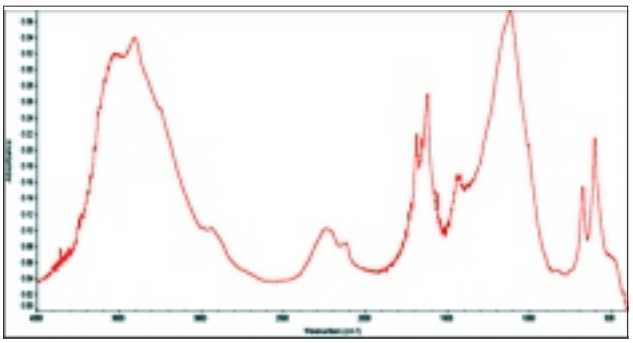

Fig. 10. *FT-IR spectrum of fucoidan*

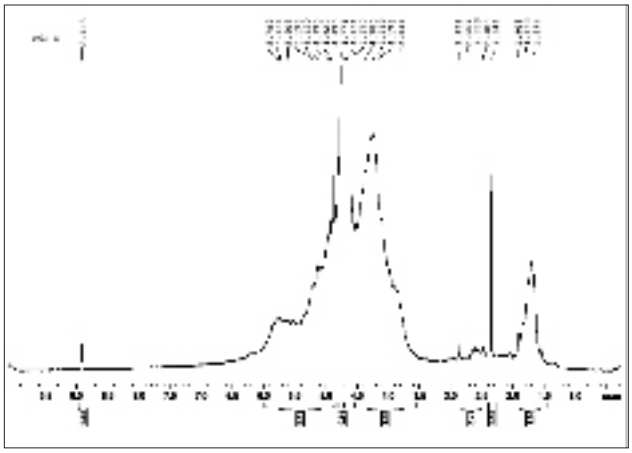

Fig. 11. *^{1}H NMR spectrum of fucoidan*

Peak at 1.694ppm represents H-6. Sharp peak at 2.2 shows the presence of O-acetyl group protons. The multiple peaks between 3.844 - 4.970 correspond to H-2 – H-5 with probable increasing deshielding order of H-4 < H-3 < H-5 < H-2. The deshielding effect in position of H-2 may show the sulphation at C-2 position (Bilan *et al.,* 2002). The presence of acetate group protons gives the probability of acetylation alternate to sulphation at C-4 position (Bilan *et al.,* 2004). Based on this comparison the observed values of the spectra may be correlated with the ring protons as: 4.870 to H-4, 4.380 to H-3, 4.721 to H-2 and 4.243 to H-5 with 1→3 linkage and sulphation at C-2 and C-4 with optional and alternate acetate group presence in the fucose unit (Karmakar *et al.,* 2009 **(**Fig. 11**)**.

Acid degradation of algin

Since the homopolymer of algin exhibits variety of biological activities (Suzuki *et al.,* 1993), several chemical and biological methods to fractionate or degrade the parent algin have been reported. The fraction/product rich in either of G or M units could be obtained in addition to reduction in molecular weight leading to oligosaccharides (Haug 1959; Heyraud *et al.,* 1996). In the present experiment degradation of algin with 1N sulphuric acid afforded products fractionated at pH levels of 2, 4, 6 and 8 with maximum yield at pH 4. The IR and ^{1}H NMR spectra were recorded and spectral data were analyzed.

IR: A representative case of IR spectra of one of the acid degraded products of algin (Fig.12) has been shown to have the values of 3475 (OH), 1670 (C=O), 1520 (COOH), 1210 (SO_3) cm^{-1} pertaining to uronic acid residues (Chandia *et al.,* 2004; Ganapathi *et al.,* 2013). With the help of characteristic bands, M and G units could be distinguished by their IR spectrum in the fractionated products. Strong bands at 800, 840 and a shoulder at 900 cm^{-1} are characteristic of C-H deformation band of β-mannuronic acid unit present in the fraction soluble at pH 2 (Mathlouthi and Koenig 1986). Bands at 790, 920 and 960 cm^{-1} are attributed to guluronic acid unit, present in fraction

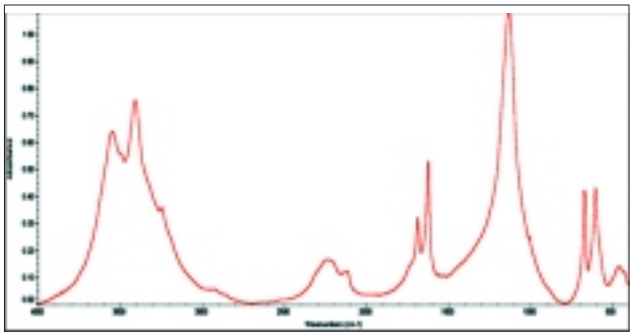

Fig. 12. *FT-IR spectrum of algin (acidic fraction)*

soluble at pH 4 of the acid degraded algin (Chandia *et al.,* 2001). Fraction of pH 6 and 8 had both G and M units with the presence of bands attributed to both M and G units of algin.

^{1}H NMR: The fractions were distinguished with the characteristic peaks of both M and G units. H-1 of G at 5.532 ppm was less prominent than the value of more prominence at 5.114 ppm pertaining to H-1 of M in the M-rich fraction obtained at pH 2 compared to the minor peak at 4.914 ppm. This is reverse in the case of fraction obtained at pH 4 with major peak H-1 of G at 5.511 and minor peak H-1 of M at 5.107. This relative abundance of M and G units could also be ascertained with the peaks of H-5 of G at 4.914 ppm with less prominence in pH 2-fraction and of H-5 of 4.908 with prominence in pH 4-fraction. For pH 6 and pH 8 fractions, G unit abundance was higher as the respective peaks were prominent and in pH 8 fraction the H-1 of M peak at 5.106 was negligence. With this observations, the abundance of M and G units in the acid degraded fractions were found out (Burana-osot *et al.,* 2009). The extent of this work will be the way forward for the unambiguous preparation of the isomer rich products which will find use in the lines of applications reported, as it is involving simple steps.

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