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Structural characterization and bioactivities of sulfated polysaccharide from *Monostroma oxyspermum*

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ABSTRACT

Sulfated polysaccharide was isolated from *Monostroma oxyspermum* through hot water extraction, anion-exchange and gel permeation column chromatography. The sulfated polysaccharide contained 92% of carbohydrate, 0% of protein, 7.8% of uronic acid, 22% of ash and 33% of moisture respectively. The elemental composition was analyzed using CHNS/O analyzer. The molecular weight of sulfated polysaccharide determined through PAGE was found to be as 55 kDa. Monosaccharides analysis revealed that sulfated polysaccharide was composed of rhamnose, fructose, galactose, xylose, and glucose. The structural features of sulfated polysaccharide were analyzed by NMR spectroscopy. Further the sulfated polysaccharide showed total antioxidant and DPPH free radical scavenging activity were as 66.29% at 250 μg/ml and 66.83% at 160 μg/ml respectively. The sulfated polysaccharide also showed ABTS scavenging ability and reducing power were as 83.88% at 125 μg/ml and 15.81% at 400 μg/ml respectively. The anticoagulant activity was determined for human plasma with respect to Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) was 20.09 IU and 1.79 IU at 25 μg/ml respectively. These results indicated that the sulfated polysaccharide from *M. oxyspermum* had potent antioxidant and anticoagulant activities.

1. Introduction

The cell walls of seaweeds are rich in matrix sulfated polysaccharides and they exhibited beneficial biological activities such as antioxidant, anticoagulant, anticancer and antiviral [1–3]. The sulfated polysaccharides are generally extracted with hot water or acid by using large volume of solvent and long extraction time [4,5]. Nowadays, researchers develop different types of extraction for polysaccharides from the seaweeds (distilled water, hot water, cold water extraction and Microwave-assisted extraction) [6]. However, seaweed cell walls and cuticles are chemically and structurally more complex and heterogeneous than those of terrestrial plants.

In recent years, algal polysaccharides have been demonstrated to play an important role as free-radical scavengers and antioxidants for the prevention of oxidative damage in living organisms [7]. Among which, the marine algae represent one of the richest natural antioxidant sources [8]. It is supposed that marine algae are protected against oxidative weakening by certain antioxidant systems. In addition, marine food processing by-products can be easily utilized to producing nutraceuticals and functional food ingredients with antioxidative property [9]. Most of the research on marine derived antioxidants has focused only on potency of crude extracts and however purification and characterization of antioxidant from such source are very limited [10].

The antioxidant activity of sulfated polysaccharides has been investigated by different in vitro methods, including hydrogen peroxide, superoxide anion, and hydroxyl radical scavenging assays. DPPH radical scavenging assay is frequently used for the analysis of food and substances obtained from natural sources [11]. The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertbutylhydroxytoluene (TBHQ). However, most of the antioxidants used have been suspected of being responsible for liver damage and carcinogenesis [12]. Thus, it is essential to

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develop and utilize effective natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases [13].

An investigation of blood anticoagulant properties of sulfated polysaccharide from marine brown algae has been reported an as alternative sources for manufacturing novel anticoagulant drugs [14]. Afterwards, various anticoagulant sulfated polysaccharides from marine algae have been isolated and characterized [15,16]. However, there are fewer reports of anticoagulant Sulfated polysaccharides reported from marine green algae compared to brown and red algae [17,18]. The anticoagulant activity of the sulfated polysaccharides has been determined by prolongation of activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) assays. Especially, polysaccharides from Monostromaceae show potent anticoagulant activity. That the active polysaccharide extracts from *Monostroma nitidum* yield a sixfold higher activity than that of heparin discovered by [19]. In addition, two different sulfated polysaccharides from *M. nitidum* and *M. latissimum* had more potent effect on the inhibition of thrombin than heparin or dermatan sulfate [20]. Keeping the importance of sulfated polysaccharide as an antioxidant and anticoagulant in mind, the present study has been attempted to find out the in vitro antioxidant and anticoagulant effects of sulfated polysaccharide derived from *Monostroma oxyspermum*.

2. Materials and methods

2.1. Seaweed collection, identification and pre-treatment

Marine alga *M. oxyspermum* of class Chlorophyceae (green algae) was collected during the low tide period from the intertidal region (up to 0.5–2 m depth) from the coastal area of Kanyakumari (Lat. 08°05′N; Long. 77°33′E) in Tamil Nadu, Southeast coast of India. The alga was identified using the standard systematic key reference followed by Umamaheswara Rao [21]. The material was washed with seawater followed by tap water and distilled water to remove the epiphytes, other mud and sand debris and then shadow dried. The dried seaweed was cut into small pieces and powdered in a mixer grinder for further extraction.

2.2. Extraction of crude polysaccharide

Extraction of polysaccharide was carried out following the procedure previously described by Seedevi et al. [22]. Briefly, 50 g of seaweed powder was dipped in 1:50 volumes (2.5 L) of distilled water and kept at room temperature for 2 h, then homogenized and refluxed at 100 °C for 2 h. After cooling, the mixture was filtered using cheesecloth; further the supernatant was separated from the algal residue by centrifugation at 6000 rpm for 20 min. The residue was further extracted thrice (three times) with double-distilled water at 100 °C for 2 h. All extracts were pooled and concentrated under reduced pressure in a rotary evaporator and dialyzed in cellulose membrane (molecular weight cut off 12,000 kDa) against distilled water for 3 successive days. The retained fraction was freeze-dried in a lyophilizer (Pneguin plus – 4 kg, Lark India) and stored in a refrigerator.

2.3. Deproteinization

The polysaccharide mixture was dissolved in distilled water, mixed with Sevag reagent containing chloroform and n-butanol ($CHCl_3$: n-BuOH) (5:1) as described in Sevag method Staub [23] as previously described by Seedevi et al. [22]. The reagent was added for six to seven times until there was no white layer formed between polysaccharide solution and Sevag reagent and no specific absorbance at 260 nm (nucleic acid) and 280 nm (protein) was detected. After removal of the Sevag reagent, the resulting polysaccharide solution was precipitated by adding ethanol and the final mixture was kept in 4 °C overnight for further processing. The precipitate was collected afterwards by centrifugation at 4000 rpm for 20 min at 4 °C and was washed successively with petroleum ether, acetone and ethanol several times, then subjected to lyophilization. The yield of the crude polysaccharide (CPs) thus obtained was then calculated using the following equation [22].

2.4. Fractionation of polysaccharides

2.4.1. Ion-exchange chromatography

The crude polysaccharide was dissolved in distilled water (10 mg/10 ml), centrifuged at 6000 rpm for 10 min, and the supernatant was applied to a Q-Sepharose™ (GE- Heathcare) Fast Flow column (EX 150 mm × 50 cm) equilibrated with distilled water. The column was eluted stepwise with distilled water, with a linear gradient of 0–3 mol/NaCl at a flow rate of 0.55 ml/min and 15 fractions were collected. The collected fractions were estimated for the carbohydrate content by phenol-sulfuric acid reaction (Dubois et al. [24]). The fractions showing higher yield were pooled together, dialyzed against distilled water and then freeze-dried.

2.5. Purification of fractionated polysaccharide

2.5.1. Gel filtration chromatography

The semi-purified polysaccharide was further purified by gel permeation chromatography using a High-Resolution Sepharose 4-LB Fast Flow column (EX 100 mm × 25 cm), equilibrated with distilled water. The column was eluted stepwise with distilled water; with extend of 0.2 mol/l sodium acetate buffer at an elution rate of 0.33 ml/min. The collected fractions were estimated for the carbohydrate content by phenol-sulfuric acid reaction [24]. The fractions showing higher yield were pooled together, dialyzed against distilled water and then freeze-dried.

2.6. Analysis of biochemical composition

2.6.1. Determination total carbohydrate and protein content

The total carbohydrate content was estimated calorimetrically by the phenol-sulfuric acid method using D-glucose as a standard Dubois et al. [24]. Protein content was measured by using the Bradford method [25], using bovine serum albumin as a standard.

2.6.2. Determination of uronic acid

The carbazole reaction which is the most satisfactory method for estimating uronic acid was employed for quantification Bitter and Muir, [26]. The glucuronolactone was used as a standard. The results were expressed as % for sulfated polysaccharide.

2.6.3. Determination of ash content

The ash content of sulfated polysaccharide, which represents the total inorganic materials present, was quantified gravimetrically according the method of You et al. [27]. 0.5 g of the dried polysaccharides taken in a porcelain crucible was burnt at 600 °C for 8 h in a muffle furnace. The weight of the residue, which represented the ash content, was recorded and the results were given as percentage of the dry weight of polysaccharides.

2.6.4. Determination of moisture content

The moisture content of the crude and purified polysaccharides was determined by drying following the method of You et al. [27]. 0.5 mg of sample was dried in a micro oven in 130 °C for 2 h.

2.6.5. CHN/S analysis

The percentage content of carbon, hydrogen, nitrogen and sulfur of sulfated polysaccharide of *M. oxyspermum* was estimated by using the operating mode of PE 2400 series II CHNS/O analyzer EA1112 (CE Instrument, Italy). Approximately 1 mg of sample was injected, eluted with TCD (Thermal conductivity detector, CE Instrument). 2,5-Bis (5′-tert-butyl-2-benzoxazol-2-yl) thiophene (BBOT) and aspartic acid were used as reference standard.

2.7. Determination of molecular weight (PAGE)

The molecular weight of the sulfated polysaccharide was estimated by PAGE according to the method of Nikapitiya et al. [28]. 10 μg of the sample was applied to a 6% polyacrylamide gel (1 mm thickness) slab in 0.02 M sodium barbital (pH 8.6) and run for 30 min at 100 V. After that, the gel was stained with 0.1% toluidine blue in 1% acetic acid and washed in 1% acetic acid for 4 h. The Chondrioitin sulfate and Dextran sulfate (Sigma) were used as the standards. The molecular weight of the bands was calculated using Total lab software (version 1.11).

2.8. Spectroscopic analysis

2.8.1. HPLC analysis for monosaccharide content

The purified sulfated polysaccharide was dissolved in 1 ml of distilled water and mixed with an equal volume of 4.0 M trifluoroacetic acid (TFA). Samples were allowed to stand for 4 h at 100 °C and the acid-hydrolyzed sample was filtered through 0.45 μm syringe filter and the residual acid was removed through decompression and distillation with methanol for three times [29]. The sample was injected in high performance liquid chromatography (HPLC) system, (Rheodyne, Rohnert Park, CA, USA, Model 7010) with a 20 μl sample loop, a column (carbohydrate analysis column, 4.6 × 250 mm, Waters), with refractive index (RI) detector. The following neutral mono sugars were used as references: rhamnose, xylose, mannose, galactose, Fructose and glucose (Sigma).

2.8.2. FT-IR analysis

The freeze-dried samples of crude and purified polysaccharide were relied on a Bio-Rad FT-IR – 40 (USA). Each sample (10 mg) was mixed with 100 mg of dried potassium bromide (KBr) and compressed to prepare as a salt disc (10 mm diameter) for reading the spectrum further. Spectra were taken between wave numbers of 4000 and 500 cm^{-1}.

2.8.3. ^1H NMR analysis

^1H NMR spectrum of sulfated polysaccharide of *M. oxyspermum* was taken following the method of Luo and Fan [30]. Approximately 30 mg of sample was dissolved in 0.5 ml of D_2O (99.9%) in a NMR tube (5 mm diameter). The ^1H NMR spectra were taken at 27 °C and the chemical shift was expressed in parts per million (ppm).

2.9. Antioxidant activity

2.9.1. Total antioxidant activity

The total antioxidant activity was carried out according to the method described by Arivuselvan et al. [31]. Briefly, 2.0 ml of sample at various concentrations (10–160 μg/ml) was mixed with 1.0 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 90 min under water bath. After the mixture had been cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The L-ascorbic acid and BHT were used as standards and the total antioxidant capacity was expressed as ascorbic acid equivalent.

2.9.2. Scavenging ability on DPPH radicals

The DPPH free radical scavenging activity of sulfated polysaccharide was determined by following the method of Zhang et al. [32]. Briefly 0.1 mM solution of DPPH was prepared in 100% methanol, and 1 ml of this solution was added to 4 ml of sample in 40% methanol at various concentrations (10–160 μg/ml). The mixture was shaken vigorously and incubated for 15 min at 30 °C in the dark. The reduction of the DPPH radical was measured by continuous monitoring of the decrease of absorption at 517 nm. The L-ascorbic acid and BHT were used as standards and the DPPH scavenging effect was calculated as follows:

$$\text{DPPH scavenging effect}\,(\%) = 1 - \frac{\Delta\text{A sample} - \Delta\text{A blank}}{\Delta\text{A control}} \times 100$$

2.9.3. Scavenging ability on ABTS radicals

The radical scavenging capacity of sulfated polysaccharide was evaluated against ABTS radical cations generated by using the methodology of Giao et al. [33]. This method is based on the ability of antioxidant molecules to quench the long-lived $ABTS^+$ species, a blue-green chromophore with a characteristic absorption at 734 nm. The addition of antioxidants to the preformed radical cation reduces it to ABTS, causing loss of color. Results were expressed by plotting % of inhibition and L-ascorbic acid and BHT were used as standards.

$$\text{ABTS scavenging effect}\,(\%) = 1 - \frac{\Delta\text{A sample} - \Delta\text{A blank}}{\Delta\text{A control}} \times 100$$

2.9.4. Reducing power assay

The reducing power was determined as described by Zhang et al. [32]. Briefly, 1 ml of polysaccharide sample solution (10–160 μg/ml) in phosphate buffer (0.2 M pH 6.6) was mixed with 1 ml of potassium ferricyanide (1%, w/v) and incubated at 50 °C for 20 min. Afterwards 2.0 ml of TCA (10% w/v) was added to the mixture to terminate the reaction. The solution was mixed with 1.25 ml of ferric chloride (0.1% w/v) and the absorbance was measured at 700 nm. The L-ascorbic acid and BHT were used as standards.

2.10. Anticoagulant activity

2.10.1. APTT assay

Anticoagulant activity was determined by activated partial thromboplastin time (APTT) coagulation assay. Briefly, citrated normal human plasma (90 μl) was mixed with 10 μl of sulfated polysaccharide and incubated for 1 min at 37 °C in separate glass vial. The APTT and PT measurements were performed using a kit obtained from Instrumentation Laboratory (Lexington, USA). Then, bovine cephalin (100 μl) was then added and incubated at 37 °C. After 3 min of incubation, 100 μl of pre-warmed 0.25 M $CaCl_2$ solution was added to the mixture and the clotting time was measured and compared with standard, the activity was expressed as IU/mg.

2.10.2. PT assay

The Prothrombin Time (PT) assay was performed for sulfated polysaccharide. For the PT assay, citrated normal human plasma (90 μl) was mixed with 10 μl of sulfated polysaccharide and incubated for 10 min at 37 °C. After the incubation period, the pre-incubated PT assay reagent (200 μl) was added and clotting time was recorded.

2.11. Statistical analysis

All data are expressed as means ± SD. Data were analyzed by analysis of variance ($P < 0.05$) and the means separated by Duncan's

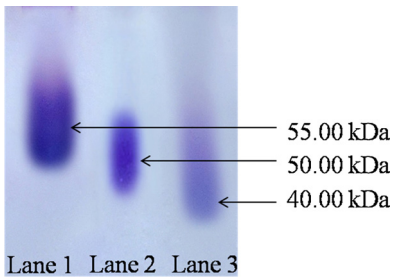

Fig. 1. Gel profile of the polyacrylamide gel electrophoresis.
Lane 1 – sulfated polysaccharide, Lane 2 – chondrioitin sulfate and Lane 3 – dextran sulfate.

multiple range tests. The results were processed using MS-Excel and SPSS version 16.

3. Results and discussion

3.1. Structural characterization

3.1.1. Yield and biochemical and elemental composition of sulfated polysaccharide

The yield of the sulfated polysaccharide from *M. oxyspermum* was found to be 18.39% (w/w) on dry weight basis. The total carbohydrate content of the sulfated polysaccharide of *M. oxyspermum* was estimated as 91.6%. The protein content of sulfated polysaccharide showed a negative response in the Bradford test, indicating the absence of protein. The ash and moisture content of the sulfated polysaccharide was reported as 16.7 and 2.9% respectively. In our previous report, the yield, carbohydrate, protein, ash and moisture content of sulfated polysaccharide of *Codium tomentosum* was found to be 10.83%, 88.7%, 0%, 15.9% and 2.3% respectively [22]. In the present study, the carbon, hydrogen, nitrogen and sulfur content of sulfated polysaccharide were 35.16%, 6.32%, 10.2% and 17.8%, respectively. In our previous report, the sulfated polysaccharide from *C. tomentosum* showed carbon (25.86%), hydrogen (5.80%), nitrogen (6.50%) and sulphur (0.16%) respectively [22].

3.1.2. Molecular weight determination of sulfated polysaccharide

The molecular weight of sulfated polysaccharide determined through polyacrylamide gel electrophoresis (PAGE) was 55 kDa (Lane 1) which were compared to the standards-Chondrioitin sulfate (50 kDa) (Lane 2) and Dextran sulfate (40 kDa) (Lane 3) (Fig. 1). In other previous report, the molecular weight of sulfated polysaccharide fractions (F1, F2 and F3) from *Sargassum plagiophyllum* was found to be 30, 35 and 20 kDa respectively [2].

3.1.3. Monosaccharide composition of sulfated polysaccharide

The Monosaccharide composition of the sulfated polysaccharide from *M. oxyspermum* revealed that rhamnose (69.32%) was the major sugar. Remaining monosugars such as Fructose (7.80%), Galactose (2.33%), Xylose (3.01%) and Glucose (1.99%) were found in minimum amounts which were compared to the standard monosugars. Likewise, the monosaccharide content of the purified sulfated polysaccharide and five de-sulfated polysaccharide from *M. oxyspermum* recorded higher amount of rhamnose (78.65–85.77%) and low amount of glucose (7.29–11.49%), xylose (4.79–8.60%), mannose (1.43–2.03%) and galactose (1.22–2.87%) [17].

3.1.4. FT-IR spectrum of sulfated polysaccharide

In the FT-IR spectrum of sulfated polysaccharide of *M. oxyspermum* the carbohydrate band started from 3425.58 cm^{-1} and down to 586.36 cm^{-1}. The hydroxyl group was represented by a band at 3425.58 cm^{-1} and residual moisture in the sulfated polysaccharide and the methylene group at 2929.82 cm^{-1} (Fig. 2). At the same time the anion carbohydrate group was represented by a band at 1620.21 cm^{-1} and α-CH_3 group at 1257.59 cm^{-1}. A characteristic absorption at 846.75 cm^{-1} was also observed, indicating the α-configuration of the sugar units [22].

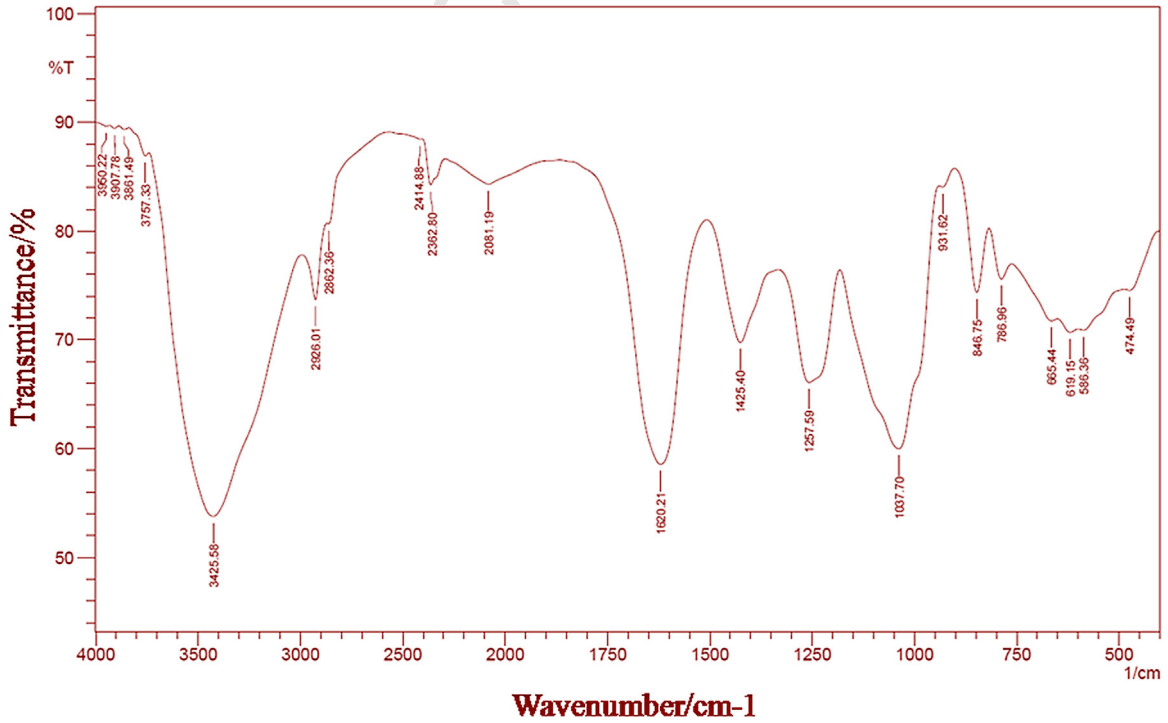

Fig. 2. FT-IR spectrum of sulfated polysaccharide from *M. oxyspermum*.

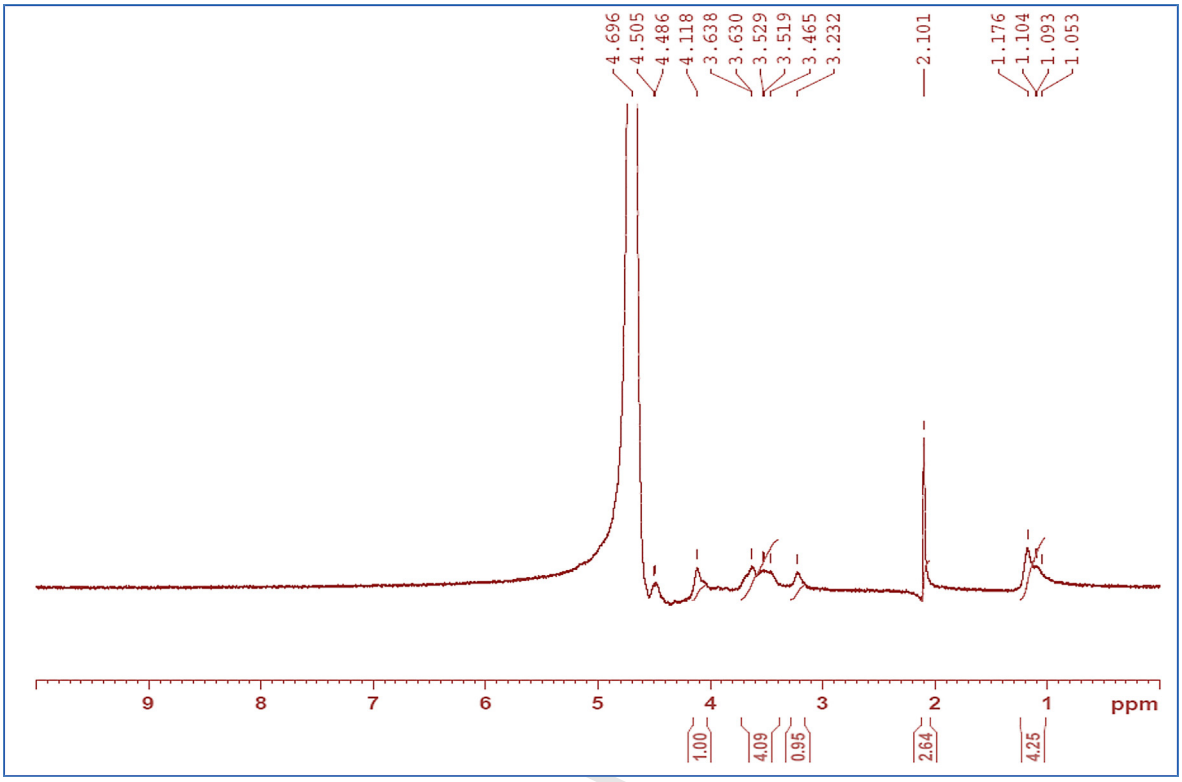

Fig. 3. ^{1}H NMR spectrum of sulfated polysaccharide from *M. oxyspermum*.

3.1.5. *^{1}H NMR spectrum of sulfated polysaccharide*

In ^{1}H NMR spectrum, the signal at 4.118–4.505 ppm showed (β-configuration) β-D-galactose (G) was linked to α-L-galactose 6-sulfate and that of β-D-Galactose (G) was linked to 3,6-α-L-anhydrogalactose in the sulfated polysaccharide from *M. oxyspermum* (Fig. 3). The signal at 3.683–3.765 ppm represented α-L-rhamnopyranosyl residues and the signal at 2.101 ppm indicates the sharp singlet at single proton which indicates α-carbon proton. The signals of the last region at 1.053 and 1.176 ppm were assigned to C6 methyl protons of L-fuco- pyranose.

3.2. Total antioxidant activity

The total antioxidant capacity (TAC) assay allows determination of the antioxidant potential of natural compounds. In the present study, the sulfated polysaccharide showed the total antioxidant activity in the range of 33.2–66.29% respectively at different concentrations (50–250 μg/ml (Fig. 4)). The maximum of 66.29% inhibition was observed at the concentration of 250 μg/ml, it was observed that the total antioxidant activity was found increasing with increasing concentration. On comparison the standards BHT and L-ascorbic acid reported 90.16 and 88.15% of total antioxidant activity at the highest concentration of 250 μg/ml respectively. The sulfated polysaccharide of *M. oxyspermum* showed higher total antioxidant activity at lowest concentration than that of total antioxidant activity of polysaccharide from *S. tenerrimum* – 62.55% at the maximum concentration of 1000 μg/ml [34].

3.3. The DPPH free radical scavenging

The DPPH free radical scavenging model can be used to evaluate the antioxidant activities in a relatively short amount of time. The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants through the donation of hydrogen to form the stable DPPH–H molecule [35]. Yuan et al. [36] also claimed that the model of scavenging DPPH radical is a widely used method to evaluate the free radical scavenging activities of antioxidants. DPPH is one of the compounds that possesses a proton free radical and shows a characteristic absorption at 517 nm (purple) [37]. On the basis of this principle, in the present study inhibitory effect sulfated polysaccharide on DPPH radical was found marked and concentration dependent i.e., the 10–160 μg/ml concentration showed 22.39–68.19% of inhibition respectively (Fig. 5). The highest of 66.83% was observed at the concentration of 160 μg/ml. The results showed a constant increase of DPPH antioxidant activity with increased concentration. At highest concentration, the standards BHT and L-ascorbic acid showed the antioxidant activity of 89.55% and 86.19% respectively. The DPPH scavenging effects of sulfated polysaccharide fraction (HEP and SEP) from *Enteromorpha linza* was 97.7% and 84.4% at the concentration 3.64 mg/ml [1]. The difference in the antioxidant activity of

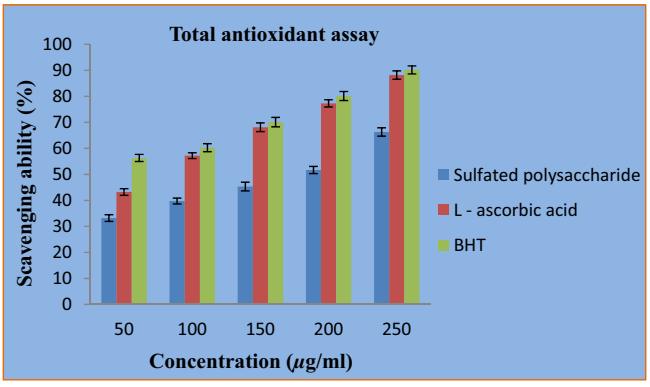

Fig. 4. Total antioxidant activity of sulfated polysaccharide.

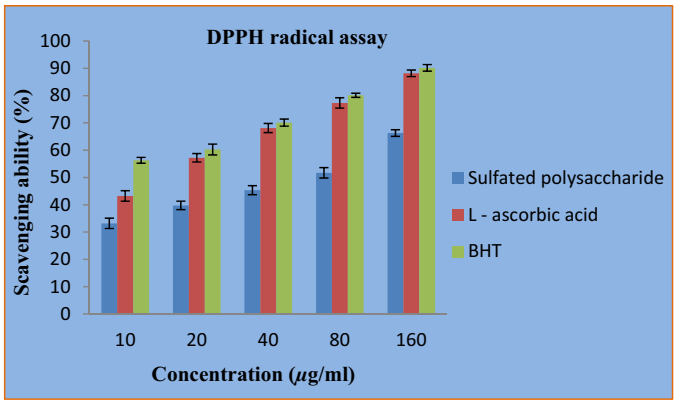

Fig. 5. DPPH antioxidant activity of sulfated polysaccharide.

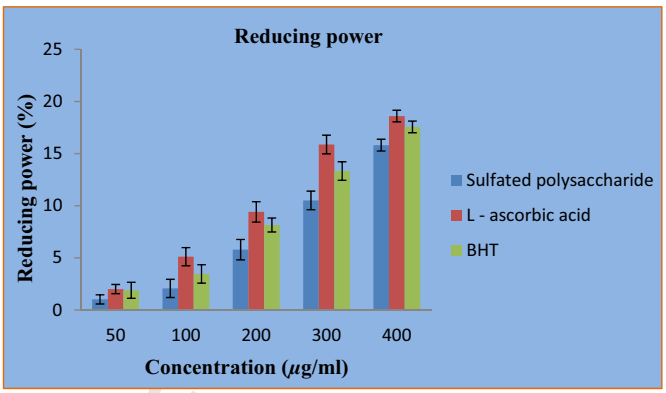

Fig. 7. Reducing power of sulfated polysaccharide.

seaweed polysaccharides may be due to their influence of lots of factors, such as the structure, molecular weight (MW) and chemical composition [32].

3.4. ABTS

ABTS (2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical is oxidised to the radical cation ($ABTS^{*+}$), its decolorization being used to measure the antioxidant capacity in both water-soluble and lipid-soluble food samples and is expressed as TEAC (Trol-ox equivalent antioxidant capacity) [11]. This activity was reported for commercial algal products [38]. In the scavenging activity on ABTS assay, we employed a specific absorbance (734 nm) at a wavelength remote from the visible region in a short reaction time, and it can be used in both organic and aqueous solvent systems and can also be an index reflecting the antioxidant activity of the test samples [21]. In the present study, the scavenging ability of sulfated polysaccharide on ABTS free radical was shown in Fig. 6. The scavenging powers of sulfated polysaccharide correlated well with increasing concentrations. The scavenging ability was reported to be 25.37–76.81% for the various concentrations 25–125 μg/ml. It showed outstanding ABTS scavenging ability, with increased concentration, whereas the standards BHT and L-ascorbic acid showed 79.39% and 83.88% of scavenging ability at the highest concentration of 125 μg/ml respectively. The result of the present study sulfated polysaccharide reported higher ABTS scavenging ability with lowest concentration, when compared the four sulfated polysaccharide fractions (UFP1, UFP2, UFP3 and UFP4) from *Ulva fasciata* reported 47.38%, 99.62%, 99.07% and 77.07% at 10 mg/ml concentration [39].

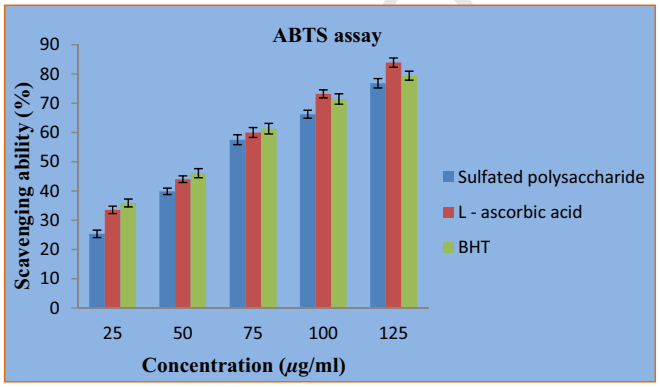

Fig. 6. ABTS radical scavenging activity of sulfated polysaccharide.

3.5. Reducing power

In the reaction system, antioxidant substances in samples cause reduction of Fe3+/ferricyanide complex to the Fe2+ form. Therefore, Fe2+ can be monitored by measuring the formation of Prussian blue at 700 nm [40]. Higher absorbance value means stronger reducing power of sample. Fig. 7 depicts the reducing powers of sulfated polysaccharide exhibited concentration dependent. The reducing power of sulfated polysaccharide was 1.02–15.81% at 50–400 μg/ml concentration, which showed a constant increase in its reducing power with increased concentration. Whereas the standards BHT and L-ascorbic acid accounted 18.6% and 17.56% of reducing power at the highest concentration of 400 μg/ml respectively. The present study sulfated polysaccharide recorded higher reducing power capacity than that of the sulfated polysaccharide fraction (UFP2 and UFP3) from *U. fasciata* which reported 1.048 and 0.612 at 10 mg/ml [39]. The reducing power was generally associated with the presence of reductones, which had been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones were also reported to react with certain precursors of peroxide, thus preventing peroxide formation [41]. The results suggested that all the samples might supply reducing agents or groups to enhance their antioxidant activities.

3.6. In vitro anticoagulant activity

The APTT and PT activity of sulfated polysaccharide was reported 7.13 IU and 1.26 IU at 25 μg/ml respectively. This might be due to the presence of sulphates groups in the polysaccharide. In fact, the sulphate content of monosaccharides of algae influences the anticoagulant activity [42]. The anticoagulant activity of the sulfated polysaccharides depends on their degree of substitution, their molecular weights and the position of the sulfate group [43]. In positive control, heparin could apparently prolong APTT and PT. From these data, the anticoagulant activities of sulfated derivatives strongly depended on their degree of sulfation, the molecular and the distribution of the sulfated group.

4. Conclusion

The sulfated polysaccharide from green alga *M. oxyspermum* was effective in vitro as an antioxidant and anticoagulant agent. These results suggest that *M. oxyspermum* may be a promising antithrombotic agent for the treatment of various thrombotic diseases in the food and pharmaceutical industries.

Acknowledgments

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