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EXTRACTION OF SULFATED POLYSACCHARIDE FROM MARINE MACRO-ALGA *Gracilaria edulis* AND ITS BIOLOGICAL APPLICATIONS

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ABSTRACT

In present study, a red macroalga *Gracilaria edulis,* collected from Rameshwaram Tamil Nadu (India) was used to extract sulfated polysaccharide by using cold acidic extraction method. The yield of sulfated polysaccharide was 2.35 g from 10 g dry powdered *G. edulis.* The extracted sulfated polysaccharide was analysed for antibacterial, anticancer, antioxidant and anticoagulant activities. The antibacterial activity of crude sulfated polysaccharide was examined on three pathogenic bacteria *viz., Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* using well diffusion method. The zone of inhibition was 20 ± 0.05 mm in case of *S. aureus,* followed by *E. coli* and *K. pneumoniae* with zone of inhibitions of 15 ± 0.04 and 12 ±0.02 mm, respectively, at 50 µg mL^{-1} sulfated polysaccharide. The anticancer activity was performed using HeLa cancer cell line and inhibitory concentration (IC_{50}) was 57.1 µg mL^{-1} as per MTT assay method. The antioxidant activity was analysed by performing 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay and the inhibitory concentration (IC_{50}) value of 174.62 µg mL^{-1} was observed. The anticoagulant assay was tested and partial thromboplastin time (PTT) increased by 4.5 sec at 40 µg mL^{-1} of sulfated polysaccharide using activated partial thromboplastin time (APTT) assay method.

Keywords: Antibacterial activity, anticancer, antioxidant, *Gracilaria edulis,* sulfated polysaccharide

INTRODUCTION

Marine macroalgae are important source of bioactive compounds with various applications in food, pharmaceutical, and cosmetic industries due to their unique chemical compositions (Saidani *et al*., 2021). Among the bioactive compounds present in macroalgae, sulphated polysaccharides have attracted significant attention due to their wide range biological activities, including anticoagulant, antiviral, and immunomodulatory properties (Bhuyar *et al.,* 2021; Karthick *et al.,* 2021). Several red, brown and green macroalgal species reportedly contain sulphated polysaccharides (Meinita *et al.,* 2021) and many macroalgae species including *Gracilaria edulis* and *Sargassum wightii* have not yet extensively been studied for their sulphated polysaccharide content. These two macroalgae species are widely distributed in tropical and subtropical regions and have traditionally been used as food and medicine (Li *et al.,* 2020). However, their potential as a source of sulphated polysaccharides has not been fully explored. Very less efforts have been made to isolate and evaluate potential biological applications of sulphated polysaccharides from *G. edulis* and *S. wightii* (Chaluvanahalli *et al.,* 2021; Wang *et al.,* 2021). Chen *et al.* (2019) observed anticancer activities of sulfated polysaccharide from

algae *Tribonema* sp. against HepG2 cancer cells; and found IC_{50} concentration at 250 µg mL^{-1}. Arunkumar *et al.* (2021) found that the sulfated polysaccharides extracted from five macroalga *viz*., *Portieria hornemannii*, *Spyridia hypnoides, Asparagopsis taxiformis, Centroceras clavulatum* and *Padina pavonica* showed antiproliferative activity against HeLa cell lines at IC_{50} value of 10 µg mL^{-1}. The present study provides a novel insight into the potential use of these two macroalgae species as a source of bioactive compounds. The present study was aimed to extract the sulfated polysaccharide, a potential bioactive compound, from *Gracilaria edulis* and and *Sargassum wightii;* and also analyse the biological activities *viz*., antimicrobial, antioxidant, anticoagulant and anticancer properties of sulphated polysaccharide isolated from *G. edulis.*

MATERIALS AND METHODS

Sample collection

The marine macroalgae samples were collected from different places of Rameshwaram marine region (Bay of Bengal), Tamil Nadu (India) using phytoplankton net (0.2 µm size). The collected red macroalgae were separated, transferred to sterile containers, transported to the laboratory and stored at 4°C for further studies. The samples were washed with distilled water and the epiphytes/ unwanted extraneous materials were removed. macroalgae were identified on the basis of their morphological characteristics and identity was confirmed by comparing the algae with a field manual (Dhargalkar and Kavlekar, 2004). The macroalgae were dried at room temperature for 5 days and finely ground in a laboratory mixer grinder. The powdered dried algae were stored in sterile plastic containers at 4°C and used when desired.

Extraction of sulfated polysaccharide

The sulfated polysaccharide was extracted from dried macroalgal powder using cold acidic extraction method (Arunkumar *et al.,* 2021). Briefly, 50 g dried powder of each marine macroalga was separately soaked in a mixture of solvent containing acetone and methanol (7:3 ratio) in glass beakers. The mixtures were incubated for 48 h in a rotary shake incubator (Subzero and Szisba model) to remove unwanted pigments and other lipophilic components. After incubation, the solvent content in the preparation was allowed to evaporate and dried biomass was further treated with 0.1N HCl and kept in a magnetic stirrer for 24 h. The process was repeated 2-3 times and the filtrate kept at 4°C for overnight. The filtrate was then precipitated by mixing with equal volume of absolute ethanol. The ethanol was evaporated and the precipitate lyophilized using a freeze dryer (Sub-zero and SZ-042BFM model). The content of extracted sulfated polysaccharide from each macroalgae was determined spectrophotometrically using gelatin-barium chloride turbidimetric method (Hemalatha *et al.,* 2021). The lipid, protein, and lipid contents were also estimated spectrophotometrically (Hemalatha *et al.,* 2021).

Antibacterial activity of sulfated polysaccharide

The extracted crude sulfated polysaccharide from G. was assessed for antibacterial activity against three pathogenic bacteria namely *Escherichia coli* (MTCC 8739), *Klebsiella pneumoniae* (MTCC 2719) and *Staphylococcus aureus* (MTCC 29736) using agar well diffusion method (Balakumaran *et al.,* 2016). The sulfated polysaccharide at different concentrations were loaded separately into each well of Petri-plates, along with chloramphenicol (30 µg mL^{-1}) as positive control for each bacterial strain. After inoculation, the plates were incubated at 37°C for 24 h; and the zone of inhibition measured in mm. In these assays, three replicates were maintained for each treatment.

MTT assay of sulfated polysaccharides

The extracted sulfated polysaccharide was subjected to cytotoxic analysis against Vero and HeLa cells (ATCC-CCL-2) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT

assay) [Balakumaran *et al.*, 2015]. The HeLa and Vero cell lines were purchased from ATCC Culture Center, USA. Both Vero and HeLa cells were separately seeded (1.4×10^4 cells $well^{-1}$) into sterile 96-well flat bottom plates and different concentrations of sulfated polysaccharide (10 to 100 µg mL^{-1}) along with doxorubicin (5 to 25 µg mL^{-1}) [TC420, Himedia] were added. The plates were incubated for 48 h at 37°C and then 150 µL of 500 mg mL^{-1} MTT solution was added to each well and incubated for 4 h. After incubation, the plates were centrifuged at 8,000 rpm for 10 min and the supernatant was removed. Then 150 µL dimethyl sulfoxide [DMSO] were added to each well and the plates were assayed using ELISA reader [BioTek Epoch2] at 570 nm. The values were recorded and percentage of cell viability calculated using the following formula (Souza *et al.*, 2018):

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \text{ x } 100$$

All the experiments were performed in triplicates and the data were expressed as mean ± standard deviation. IC_{50} value was determined using GraphPad prism 6.0. The amount of viable (living) cells is directly related to the amount of purple formazan produced by mitochondrial reductase enzymes. In order to determine the effectiveness of sulfated polysaccharide, dose-response curves were used. The purple solution was spectrophotometrically (Thermo Fisher Scientific Evolution 201) measured. The effect has been assessed based on IC_{50} values and cell survival rates (Souza *et al.*, 2018).

Acridine orange-ethidium bromide (AO-EB) staining

The extent of apoptosis of tumour cells due to the sulfated polysaccharides was assessed using AO-EB dual staining method (Souza *et al.*, 2018). Briefly, the HeLa cells were seeded (1×10^4 cells $well^{-1}$) in 24 well cell culture plates and incubated at 37°C in a cell culture incubator for 24 h with 5% supply of CO_2. The cells were treated with IC_{50} concentration of sulfated polysaccharide of macroalga *G. edulis* and incubated for 24 h. After incubation, the cells were washed twice with phosphate buffered saline (PBS) and 10 µL acridine orange and ethidium bromide stain mixture (1 mg mL^{-1} each in PBS) and kept for 5 min at room temperature. The cells were washed again with PBS buffer so as to remove the excessive stain. The cells were then visualized using FLoid® cell imaging station (Life Technologies) with an excitation filter of 482 nm for the presence of apoptotic cells.

ABTS activity of sulfated polysaccharides

The antioxidant property of sulfated polysaccharide (from *G. edulis*) was evaluated by 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay (Wang *et al.*, 2021). The method works on the ability of antioxidants to inhibit ABTS, a cationic radical in environment, thus forming a grey/blue coloured chromophore substance which can be measured at 734 nm. In present study, ABTS cationic radical solution was developed by treating 7 mM of aqueous ABTS solution with 2.45 mM potassium persulfated (final concentration); and the preparation was kept in dark at room temperature for 12-16 h. Prior to analysis, the ABTS solution was diluted using ethanol to obtain an absorbance value of 0.700 ± 0.005 at 734 nm. Different concentrations of sulfated polysaccharide varying from 100 to 500 µg mL^{-1} was prepared. Two mL of different concentrations of sulfated polysaccharide was mixed with 5 mL ABTS radical solution and allowed to react for 6 min at room temperature. After incubation, the absorbance of each sample was measured at 734 nm (BioMate 160 UV-Vis, Thermo Scientific). The reaction mixture containing ABTS without samples served as control. The ability of sulfated polysaccharide to scavenge ABTS radicals was determined using the following equation and expressed as percentage of inhibition (Wang *et al.*, 2021):

$$\text{\% inhibition} = \frac{(\text{Abc} - \text{Abs})}{\text{Abc}} \text{ x } 100$$

where, Ab_c is the absorbance of control and Ab_s is the absorbance of sample

In vitro anticoagulant assay

The *in vitro* anticoagulant property of sulfated polysaccharides was assessed as per APTT assay (Saidani *et al.*, 2021). Prior to the analysis, the human plasma was separated using standard method and the reagents such as APTT assay reagent, PT assay reagent and TT assay reagent kept at 37°C for

10 min. Then to 90 µL human plasma (citrated), 10 µL of varying concentrations of sulfated polysaccharides (0, 10, 25, 50, and 100 µg mL^{-1}) was added and the preparation incubated for 1 min at 37°C. To this, 100 µL of pre-incubated APTT assay reagent was mixed and further incubated for 2 min at 37°C. Then 100 µL of pre-warmed 0.25 M calcium chloride was added to the mixture and clotting time was monitored. Similarly, for PT assay 90 µL human plasma (citrated) was mixed with 10 µL of varying concentrations of sulfated polysaccharides (0, 10, 25, 50, and 100 µg mL^{-1}). The mixture was incubated for 1 min at 37°C, then 200 µL of pre-incubated PT assay reagent added and clotting time was monitored. For TT assay, 90 µL human plasma (citrated) was mixed with 10 µL of varying concentrations of sulfated polysaccharides (0, 10, 25, 50, and 100 µg mL^{-1}). The preparation mixture was incubated for 1 min at 37 °C, and then 200 µL of pre-incubated TT assay reagent was added and clotting time was monitored. The reaction mixture containing saline solution (0.9% NaCl) was used as negative control and heparin was used as positive control for the experimental comparison of anticoagulant activity of sulfated polysaccharide (Jing *et al.*, 2018).

Statistical analysis

All the experiments were performed in triplicates and the data were expressed as mean ± standard deviation. The T-test was performed at $P \leq 0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Sample collection and identification of strains

The macroalgae were collected from Rameshwaram marine region (Bay of Bengal), Tamil Nadu, India. Two macroalgae were identified based on their morphological characteristics. The macroalga exhibiting reddish-brown colour, discoid holdfast, cylindrical thallus, 5-10 cm long, irregular growth, arcuate branches, discoid holdfast, etc. was identified as *Gracilaria edulis* (Gmelin) and the alga having brown colour, holdfast, stipe and frond, branched thallus and berry-like structure pneumatocysts was identified as *Sargassum wightii* (Greville). The identity of *G. edulis* was in agreement with those of Yang *et al.* (2012), Meinita *et al.* (2021) and Susanti *et al.* (2022). The identity of *Sargassum wightii* was in line with Chaluvanahalli *et al.* (2021).

Extraction and estimation of sulfated polysaccharide

The sulfated polysaccharide was extracted by using cold acidic extraction method and the extracted total polysaccharide yield was 2.35 g 10 g^{-1} dry powder of *G. edulis* and 1.87 g 10 g^{-1} of *S. wightii*. The protein, carbohydrate, lipid, ash, sugar, and sulfate contents in *G. edulis* and *S. wightii* extracts are given in Table 1 and 2, respectively. The sulfated polysaccharide from macroalgae was determined spectrophotometrically using gelatin-barium chloride turbidimetric method. The extracted sulfated polysaccharide was used for further studies. Palani (2022) extracted 320 mg sulfated polysaccharide from 3 g dry *Hypnea valentiae*. Hemalatha *et al.* (2021) got sulphated polysaccharide yield of 1.95 g from *Sargassum*

Table 1: Physicochemical properties of *Gracilaria edulis* and *Sargassum wightii*

Chemical constituent	*Gracilaria edulis*	*Sargassum wightii*
Moisture (%)	78.5 ± 0.7	81.2 ± 1.1
Ash (%)	14.2 ± 0.4	22.6 ± 0.8
Proteins (%)	4.9 ± 0.3	3.1 ± 0.2
Lipids (%)	0.7 ± 0.1	1.3 ± 0.2
Carbohydrates (%)	2.2 ± 0.2	1.8 ± 0.1

Table 2: Sulphated polysaccharide yield and total sugar content of *Gracilaria edulis* and *Sargassum wightii*

Macroalgae	Sulphated polysaccharide yield (g)	Total sugar content (mg g^{-1})	Sulphate content (mg g^{-1})
Gracilaria edulis	2.35 ± 0.9	93.8 ± 0.9	17.2 ± 0.63
Sargassum wightii	1.20 ± 0.6	62.2 ± 0.45	14.9 ± 0.34

sp. by using solvent extraction method. Similarly, 0.5, 1.50 and 1.5 g sulfated polysaccharide was extracted from macroalgal species *Gracilariopsis persica* and *Ulva intestinalis* (Safavi *et al.*, 2019). Saidani *et al.* (2021) have reported that five macroalga *viz.*, *Cystoseira humilis, Padina pavonica, Halopteris scoparia, Sargassum vulgare* and *Rhodomela confervoides* yielded 8.55, 7.84, 6.43, 3.04 and 4.16 mg sulfated polysaccharide g^{-1} dry algae, respectively.

Antibacterial activity of crude sulfated polysaccharides

The potential effects of algal constituents against various diseases have attracted the attention of researchers as a new source of bioactive compounds, in addition to their nutritional properties. The extracted sulfated polysaccharide from *G. edulis* showed maximum antibacterial activity against *S. aureus*, followed by *E. coli*, and *K. pneumoniae*. The crude sulfated polysaccharide extracted from *G. edulis* showed maximum activity against *S. aureus* at the concentration of 50 µg with zone of inhibition 20 ± 0.05 mm, followed by *E. coli* with a zone of inhibition of 15 ± 0.04 mm and *K. pneumoniae* with 12 ± 0.02 mm (Table 3). The antimicrobial activity was observed against three bacterial pathogens *viz.*, *S. aureus, E. coli* and *Streptococcus sanguinis* at 500 µg mL^{-1} of sulfated polysaccharide reported by Jun *et al.* (2018). Palani *et al.* (2022) tested sulfated polysaccharide against four pathogenic bacteria *viz.*, *E. coli, E. faecalis, P. aeruginosa* and *S. aureus* and found the zone of inhibitions as 5.0 ± 0.577, 9.0 ± 1.155, 5.0 ± 0.412, 8.0 ± 1.155 at 40 µg mL^{-1}. Similarly, Jasem *et al.* (2021) reported that 50 µg mL^{-1} of sulfated polysaccharide was effective against pathogenic bacteria *S. aureus, Bacillus anthracis, Enterobacter aerogenes* and *Psudomonas aeruginosa*, and the zone of inhibition were 27.0 ± 1.0, 18.0 ± 1.2, 18.3 ± 1.4 and 19.0 ± 0.9 mm, respectively.

Table 3: Antibacterial activity of crude sulfated polysaccharide extracted from *Gracilaria edulis* using well diffusion method

Pathogenic bacteria	Zone of inhibition size (mm)			
	Control	Chloramphenicol (30 µg mL^{-1})	Sulfated polysaccharide (µg mL^{-1})	
			25	50
S. aureus	0	8 ± 0.03	14 ± 0.02	20 ± 0.05
E. coli	0	6 ± 0.02	8 ± 0.04	15 ± 0.04
K. pneumoniae	0	2 ± 0.02	7 ± 0.03	12 ± 0.02

In vitro antiproliferative activity of sulfated polysaccharide

In this study, the cancerous HeLa cells were targeted and the normal Vero cells were used as control. A colorimetric MTT test was performed on sulfated polysaccharide extracted from *G. edulis* to determine its cytotoxicity. The study revealed that when the concentration of sulfated polysaccharide increased, the cell survivability decreased. The sulphated polysaccharide showed significant activity with IC_{50} value of 57.1 µg mL^{-1} (Fig. 1; Table 2). The IC_{50} values were higher for sulfated polysaccharide against HeLa cells at lower concentrations, indicating a higher level of potency. The sulfated polysaccharide displayed dramatic morphological changes, such as more spherical shapes and shrinking cells. Under inverted

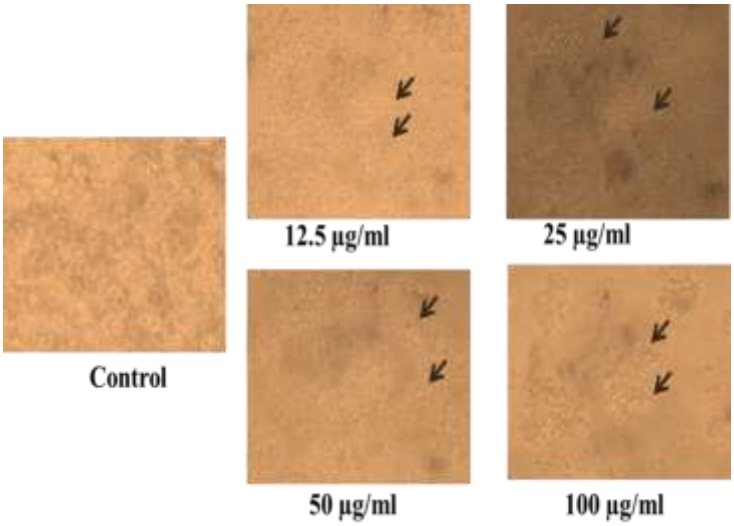

Fig. 1: Analysis of i*n vitro* antiproliferative activity against HeLa cells; ⟶ Indicates the morphological changes and cell shrinkage in the HeLa cells

Table 2: Analysis of *in vitro* anti-proliferative activity against HeLa cells and Vero cells (control)

Concentration (µg mL^{-1})	Vero cells (control)	HeLa cells
12.5	100 ± 0.12	87.59 ± 0.23
25.0	100 ± 0.11	63.17 ± 0.31
50.0	100 ± 0.10	50.45 ± 0.28
100	100 ± 0.12	27.53 ± 0.19

microscope morphological changes in cells were observed, while control cells were polygonal with short antennas, the affected cells were round. After treatment with purified sulphated polysaccharide from *G. edulis*, the cells showed huge morphological changes including shrinkage, loss of adhesion, decreased volume, rounded shapes, and sporadic distributions. Karthick *et al.* (2019) have reported that the sulfated polysaccharide extracted from *S. wightii* exhibited anticancer activity against MCF-7 cells at IC_{50} value of 39.46 mg mL^{-1}. Mendhulkar *et al.* (2020) demonstrated the anticancer activity of sulfated polysaccharide from *Spirulina platensis* against breast cancer cell line (MCF7) at IC_{50} value of 62 µg mL^{-1}. Padmanaban *et al.* (2022) observed anti-proliferative activity against breast (MDA-MB-231) and cervical (HeLa) cancer cells at IC_{50} value 350 µg mL^{-1}, liver (HepG2) cancer cells at IC_{50} value 400 µg mL^{-1} and colon (HT-29) cancer cells at IC_{50} value 200 µg mL^{-1} sulfated polysaccharide extracted from marine bivalves.

Morphological evidence for apoptosis by AO/EB dual staining

Apoptotic morphological changes were identified in HeLa cells treated with sulfated polysaccharide using AO/EB staining method. A high proliferation rate of cancer cell lines was observed when purified sulfated polysaccharide was analysed using the AO/EB staining technique. Viable and non-viable HeLa cells were stained using AO-EB after they were treated with IC_{50} amount of sulfated polysaccharides. Normal, healthy, viable cells are stained with AO stain and emit a faint green fluorescence; early apoptotic cells emit bright green fluorescence, while EB stains apoptotic cells (non-viable cells) that emit a bright red fluorescence. Condensed chromatin emits orange colour in late apoptotic HeLa cells, while red colour emanates from non-viable cells. After the treatment with sulfated polysaccharides, early apoptotic cells displayed condensed or fragmented chromatin with bright green nuclei; late apoptotic cells displayed orange chromatin with apoptotic bodies (Fig. 2).

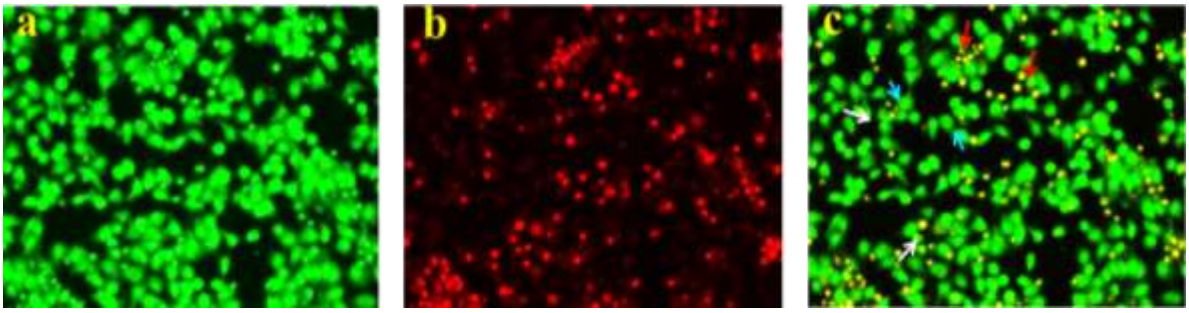

Fig. 3: Apoptosis by acridine orange/ethidium bromide (AO/EB) staining a) control (Vero cells), b) ethidium bromide (HeLa cells), and c) acridine orange (HeLa cells). Fluorescent microscopic images of sulfated polysaccharide treated HeLa cells showing apoptotic bodies and fragmented nuclei. Blue arrows indicate live cells; white arrows indicate early apoptotic cells; red arrows indicate late apopototic cells; the images were captured at a magnification of 200X. The AOEB images were analysed from the HeLa cells using IC_{50} value of 57.1 µg mL^{-1}.

ABTS radical scavenging assay

Sulfated polysaccharide extracted from *G. edulis* was measured for its antioxidant activity by its ability to scavenge ABTS free radicals and was compared with catechin as a standard antioxidant. During the treatment, ABTS and potassium persulfate react to form ABTS+ chromophore through its reaction. As measured by ABTS assay, the polysaccharide from *G. edulis* showed significant antioxidant activity with an IC_{50} value of 174.62 µg mL^{-1} (Fig. 4). The ABTS radical reactions generally include electron transfer and the reaction takes place at a faster rate when compared to

DPPH radicals. In present study, the highest antioxidant activity was observed in ABTS (55 ± 3.61%), followed by H_2O_2 (47.23 ± 2.81%) and DPPH (25.33 ± 2.52%) [Vijayabaskar *et al.*, 2012]. Seedevi *et al.* (2015) have documented the antioxidant activity of sulfated polysaccharide from *Monostroma oxyspermum* and found ABTS scavenging ability and reducing power of 83.88% at 125 µg mL^{-1} and 15.81% at 400 µg mL^{-1}, respectively. Similarly, Wang *et al.* (2022) studied the sulfated polysaccharide extracted from *Rhodosorus* sp. alga; and found ABTS radical scavenging activity at IC_{50} values of 1.74 mg mL^{-1}. Mousavian *et al.* (2022) have stated that ABTS radical scavenging activities of sulfated polysaccharide was extracted from algae at IC_{50} values of 1 mg mL^{-1}. The acid extraction process yielded polysaccharides with highest scavenging action (Peasura *et al.*, 2015).

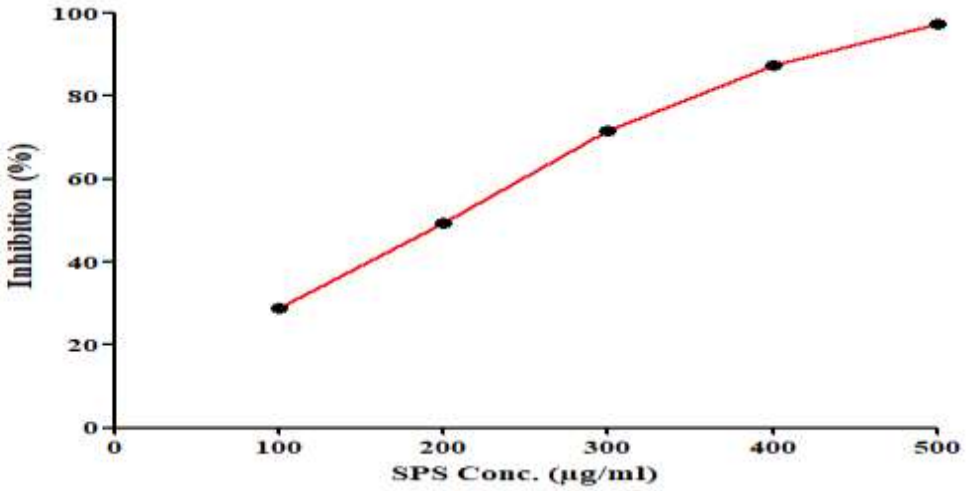

Fig. 4: ABTS free radical scavenging activity of sulfated polysaccharide extracted from *Gracilaria edulis*

In vitro anticoagulant assay

Activated partial thromboplastin time (APTT) and thrombin time (TT) techniques were used to assess the anticoagulant activity of sulfated polysaccharide extracted from *G. edulis*. The sulfated polysaccharide extracted from *G. edulis* prolonged clotting times in a dose-dependent manner. Sulfated polysaccharide extracted from *G. edulis* appears to have inhibited the intrinsic and common coagulation pathways based on their prolongation of APTT and TT. An increase in APTT and TT were observed at the concentrations > 14 g mL^{-1} of sulfated polysaccharide, while maximum prolongation of coagulation time and TT were reached at 70 and 20 g mL^{-1} of sulfated polysaccharide, respectively. The TT time was extended twice by polysaccharide at 14 g mL^{-1}, while the APTT time was increased 4.5 times by sulfated polysaccharide from *G. edulis* at 40 µg mL^{-1}. Fidelis *et al.* (2014) evaluated different extraction procedures such as sonication, enzyme digestion in combination with proteolytic extraction and found NaOH/sonication/proteolysis as the best conditions to extract anticoagulant and antioxidant SPs from *G. birdiae*. The acid extraction process yielded polysaccharides with highest scavenging action (Peasura *et al.*, 2015). Selim *et al.* (2015) examined the biological activities of sulfated polysaccharides from *Caulerpa prolifera*, and found that the extracts displayed anticoagulant activities. The antibacterial activity of kappa (ķ)-carrageenan isolated from red alga *Hypnea musciformis* was evaluated and was found effective against *Staphylococcus aureus* (Souza *et al.*, 2018). Sudharsan *et al.* (2018) assessed the anticoagulant activity of sulfated galactans from *Spyridia hypnoides* on human blood clotting by employing APTT and PT assay. In APTT test, the sulfated galactan showed anticoagulant activity of 25.36 ± 1.5 IU at 25 µg mL^{-1}; while the standard heparan sulfated demonstrated activity of 170 ±1.2 IU. The PT assay for extrinsic coagulation pathway recorded clotting at 2.46 ± 0.83 IU and 124 ± 1.0 IU for sulfated galactans and heparan sulphated, respectively.

Conclusion: The sulfated polysaccharide extract from red alga *Gracilaria edulis* possessed the antibacterial, antioxidant, anti-proliferative, and anticoagulant properties, and may be effective for treatment of variety of diseases. The study suggests that sulfated polysaccharide may be useful as a functional food ingredient or/and drug for the treatment of inflammatory diseases.

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