RESEARCH PAPER

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SULFATED POLYSACCHARIDES OBTAINED FROM THE GREEN ALGA ULVA sp.

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Abstract
Ulva sp. was collected from the Mandapam coast of Ramanathapuram, Tamil Nadu, to investigate its sulfated polysaccharides (SPs). Hot-water extraction of 100 g of dried algal material yielded 7.15 g of crude extract, and purification using DEAE-cellulose yielded approximately 4.67 g. Uronic acid and sulfate contents were estimated using standard methods, and the carbon, hydrogen, nitrogen, and sulfur contents were determined by elemental analysis. The extract showed antibacterial activity against the tested pathogens, with a reported maximum inhibition zone of 19 mm against Escherichia coli. Antioxidant activity was evaluated using standard assays, and the emulsification index (E24) was also determined.

Keywords: Ulva sp.; sulfated polysaccharides; antioxidant activity; antimicrobial activity; DPPH; ABTS; total antioxidant capacity; emulsification index

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INTRODUCTION

Seaweeds are important marine primary producers and represent a rich source of biomass (Iyapparaj et al., 2010). Marine organisms produce diverse bioactive compounds, including proteins, lipids, carbohydrates, polyphenols, minerals, and pigments. These compounds have potential applications in the food and pharmaceutical industries (Ahmed et al., 2024). Consequently, marine-derived primary and secondary metabolites have attracted increasing research interest as potential sources of bioactive products. Marine algae, in particular, have gained attention because of their nutritional and potential therapeutic properties (Harinathan et al., 2025).

Biological control of plant diseases and the use of organic materials can contribute to sustainable agriculture. Organic amendments may improve plant growth and nutrient uptake through mechanisms that include phytohormone production, nitrogen fixation, and phosphate solubilisation (Prabu et al., 2013; Shankar et al., 2013; Sankaralingam et al., 2014; Harinathan et al., 2021). Coastal physico-chemical conditions can influence the composition and productivity of marine flora and fauna. These parameters may affect species diversity, reproductive patterns, spawning, and survival (Ravichandran et al., 2022).

Previous studies have reported that contaminants can adversely affect aquatic ecosystems and biodiversity (Anand et al., 2021; Lakshmikanth et al., 2021). Along the southeastern coast of India, the Gulf of Mannar Biosphere Reserve includes biodiverse coastal areas such as Rameswaram, Mandapam, Keelakarai, Vembar, and Thoothukudi (Ravichandran et al., 2022).

Extracts of *Kappaphycus alvarezii* have been reported to contain compounds with antioxidant, anti-inflammatory, antitumour, antiviral, antidiabetic, and antihyperlipidaemic activities. Marine-derived compounds are also being investigated for other potential biomedical applications (Sharma et al., 2023; Harinathan et al., 2025).

Oxidative stress contributes to several disorders, including cancer, neurological disorders, and cardiovascular disease. Antioxidants from natural sources may help to limit oxidative damage by scavenging free radicals and reducing the oxidation of biomolecules (Liu et al., 2020). Therefore, this study investigated the antimicrobial and antioxidant activities of sulfated polysaccharides extracted from the green alga *Ulva sp.*

Materials And Methods

Isolation and extraction of sulfated polysaccharide

The algal sample was collected from the Mandapam coast in Ramanathapuram District, Tamil Nadu, India (9°16′18.2″ N, 79°10′05.04″ E). The cleaned sample was dried, and 100 g of dried material was mixed with three volumes of water and heated at 90–95°C for 16 h. The mixture was filtered through Whatman

No. 3 filter paper. The filtrate was cooled and concentrated to one-quarter of its initial volume, after which 3 L of ethanol was added to precipitate the polysaccharides.

Purification of sulfated polysaccharide

The crude extract was purified by column chromatography. The extract was dissolved in distilled water and centrifuged at 6,000 rpm for 10 min to obtain the supernatant. The supernatant was loaded onto a DEAE-cellulose column (3 × 45 cm) equilibrated with distilled water, and 50 fractions were collected. Carbohydrate content was estimated using the phenol–sulfuric acid method (Dubois et al., 1956). Fractions showing a positive response and higher yield were pooled, dialyzed against distilled water, and lyophilized. Sulfate was detected using the barium chloride–gelatin method (Lloyd et al., 1961), and uronic acid was estimated using the carbazole method (Bitter and Muir, 1962).

Analysis of elements

The carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) contents of the sulfated polysaccharide from *Ulva sp.* were determined using a CHNS/O analyser (EA1112, CE Instruments, Italy; PE 2400 Series II).

Antimicrobial activity of sulfated polysaccharides

The in-vitro antimicrobial activity of the sulfated polysaccharide extract was assessed against bacterial pathogens. The organisms include *Klebsiella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Serratia sp., Salmonella sp., *Xanthomonas sp.*, *Erwinia carotovora*, *Listeria monocytogenes*, and *Erwinia amylovora*.

Antibacterial assay

Antibacterial activity was assessed using a disc-diffusion assay, following the cited method (El-Masry et al., 2000). Whatman No. 1 filter paper was cut into 6-mm discs and sterilised at 121°C for 20 min. After the culture medium solidified, wells were prepared using a sterile well cutter. A 24-h culture was spread over the medium with a sterile cotton swab. The test sample was added to one well and distilled water was used as the control. The plates were incubated at 35°C for 24 h, after which the zones of inhibition were measured.

Free radical scavenging activity of sulfated polysaccharides from *Ulva* sp.

Evaluation of total antioxidant capacity (TAC)

Total antioxidant capacity was determined using the method of Prieto et al. (1999). Briefly, 0.3 mL of polysaccharide extract was mixed with 3.0 mL of reagent containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After incubation for 15 min, absorbance was measured at 695 nm. Ascorbic acid was used as the reference standard.

Evaluation of reducing power

Reducing power was assessed using the method of Yamaguchi et al. (1998). Different concentrations of the sample were mixed with 0.2 M phosphate buffer (pH 6.6) and 1% (w/v) potassium ferricyanide, then incubated at 50°C for 20 min. The reaction was stopped by adding trichloroacetic acid. The mixture was combined with distilled water and 0.1% (w/v) ferric chloride, and absorbance was measured at 700 nm.

Hydrogen peroxide scavenging assay

Hydrogen peroxide-scavenging activity was evaluated using the method of Gulcin et al. (2004). A 10 mM hydrogen peroxide solution was prepared in 0.1 M phosphate-buffered saline (pH 7.4). Different sample concentrations of 100, 250, 500, 750, and 1,000 µg were mixed with 2 mL of hydrogen peroxide solution. After incubation at 37°C for 10 min, absorbance was measured at 230 nm.

DPPH radical scavenging assay

The DPPH radical-scavenging assay was performed according to Blois (1958). A 0.1 mM DPPH solution was prepared in methanol and mixed (1 mL) with 3 mL of algal sample at the concentrations of 100, 250, 500, 750, and 1,000 µg. The mixture was incubated for 10 min, and absorbance was measured at 517 nm.

ABTS inhibition assay

ABTS radical-scavenging activity was assessed using the method of Re et al. (1999). ABTS solution of 7 mM, 5 mL was mixed with 88 µL of 140 mM potassium per sulfate and kept in the dark at room temperature for 16 h to generate the ABTS radical cation. The solution was diluted with ethanol as described in the original protocol, and absorbance was measured at 734 nm.

Emulsification activity

Emulsification activity was assessed using the methods attributed to Cooper and Goldenberg (1987) and Sarubbo (2006). The sulfated polysaccharide sample was tested with the oils and hydrocarbons.

Results

Yield of the crude extract

Sulfated polysaccharides were extracted from *Ulva sp.* collected at Mandapam using hot water. The extraction reportedly yielded 6.15 g of crude material from 100 g of dried algal biomass.

The crude extract was purified using DEAE-cellulose, reportedly yielding approximately 4.15 g. The carbohydrate content was 76.15 ± 0.67%. The sulfate and uronic acid contents were 16.56 ± 0.65% and 8.41 ± 0.72%, respectively, partial purification using DEAE-cellulose-52 with 1 M sodium chloride and a yield of 5 g was noted.

Elemental analysis of sulfated polysaccharides

The percentages of carbon, hydrogen, nitrogen, and sulfur in the sulfated polysaccharide extract from *Ulva sp.* are presented in Table 1.

SP extract	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)
Ulva sp.	17.4	3.6	4.5	0.11

Table 1. Elemental analysis of sulfated polysaccharides from *Ulva* sp.

Antibacterial activity of sulfated polysaccharide against pathogens

The *Ulva sp.* extract produced a inhibition zone of 7 mm against *Pseudomonas aeruginosa* and 17 mm against *E. coli* (Table: 2). Among the listed plant-associated pathogens, maximum inhibition zone of 23 mm against *Erwinia carotovora* and a minimum of 9 mm against *Xanthomonas* sp. (Table: 3).

Table 2. Antibacterial activity of sulfated polysaccharide

S. No	Test organisms	Standard (mm)	Sulfated extract (mm)
1	*Klebsiella oxytoca*	24	12
2	*E. coli*	21	17
3	*Staphylococcus aureus*	25	16
4	*Pseudomonas aeruginosa*	27	7
5	*Bacillus subtilis*	24	13
6	*Serratia* sp.	26	12
7	*Salmonella sp.*	25	14

Table 3. Antibacterial activity of SP against plant pathogens

S.No	Test organisms	Standard (mm)	Extract of SP (mm)
1	*Erwinia amylovora*	20	13
2	*Listeria monocytogenes*	18	14
3	*Xanthomonas* sp.	21	9
4	*Erwinia carotovora*	21	23
5	*Alkaligens*	25	15

Free radical scavenging activity of sulfated polysaccharides

Total antioxidant capacity

The total antioxidant capacity of the sulfated polysaccharide from *Ulva sp.*, compared with ascorbic acid, is presented in

Fig. 1. The reported activity for *Ulva sp.* was 70.25 ± 0.40%.

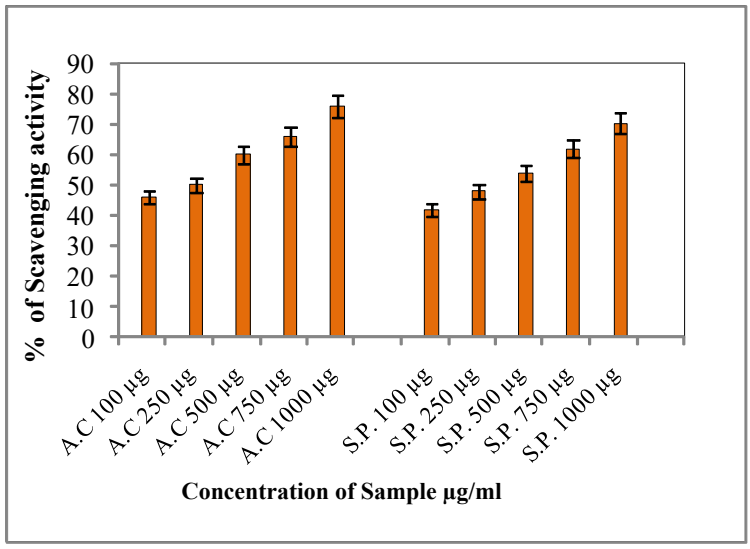


Fig. 1. Total Antioxidant capacity of sulfated polysaccharide

Reducing power

The reducing power of the sulfated polysaccharide from *Ulva sp.*, compared with ascorbic acid, is presented in Fig. 2. The obtained value was 0.992 ± 0.45.

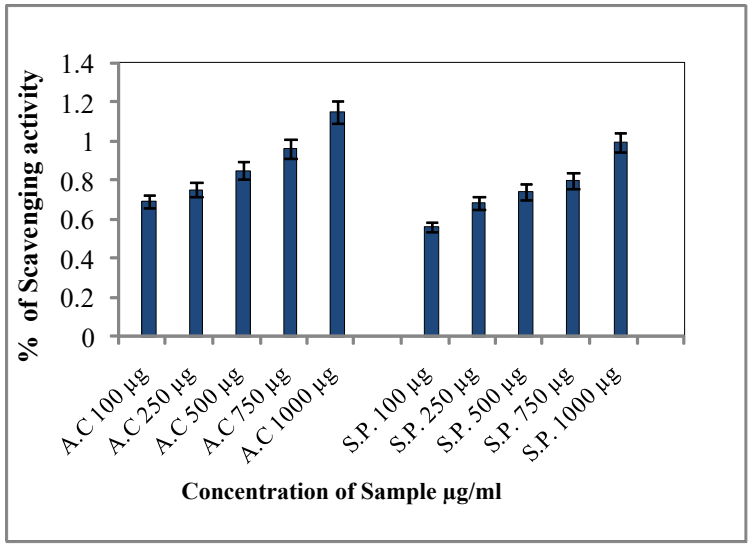


Fig. 2. Reducing power of sulfated polysaccharides

Hydrogen peroxide scavenging assay

The hydrogen peroxide-scavenging activity of the sulfated polysaccharide from *Ulva sp.* was reported as 75.32 ± 0.46%. The activity was compared with gallic acid, as shown in Fig. 3.

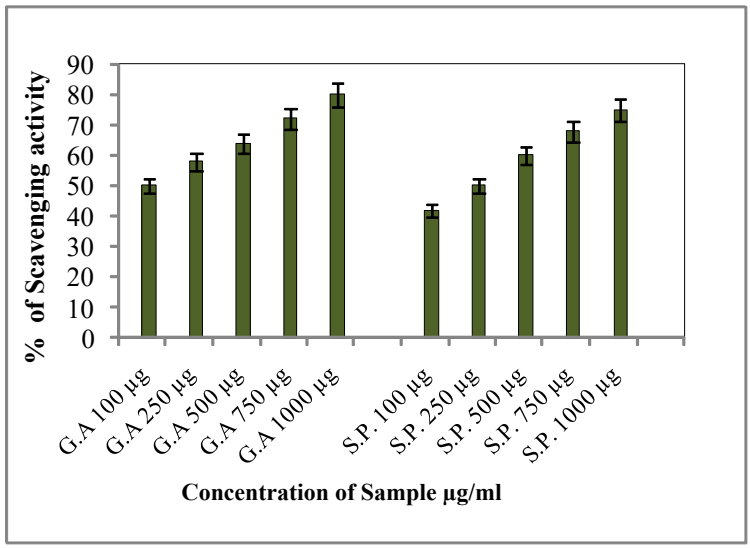


Fig. 3. Hydrogen peroxide scavenging assay of SP

DPPH radical scavenging activity

DPPH radical-scavenging activity increased with increasing sample concentration. The maximum activity was reported as 72.48 ± 0.13%, as shown in Fig. 4.

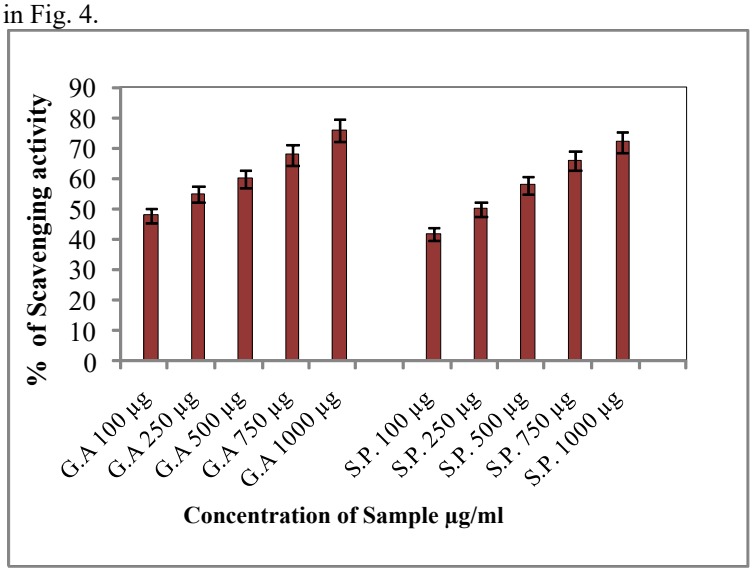


Fig.4. DPPH scavenging assay of SP

ABTS inhibition assay

The ABTS radical-scavenging activity of the sulfated polysaccharide from *Ulva sp.* was reported as 64.35 ± 0.66%. The activity increased with sample concentration, and the results were compared with gallic acid (Fig. 5).

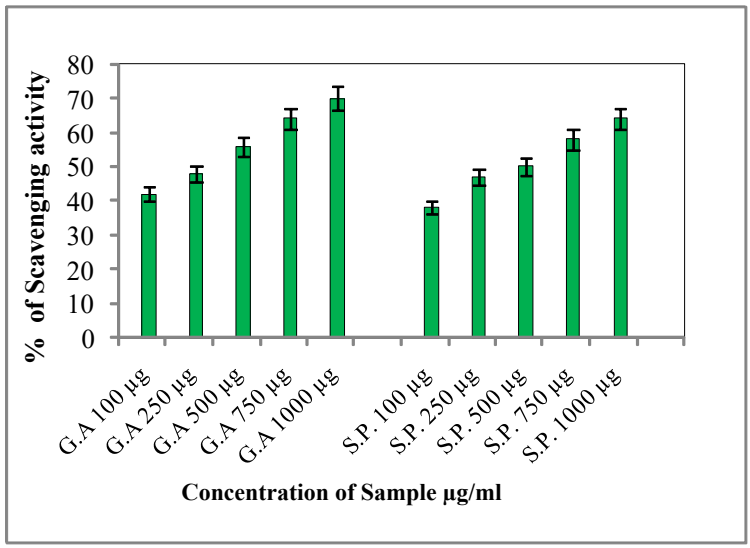


Fig. 5. ABTS scavenging assay of SP

Hydroxyl scavenging activity

The hydroxyl radical-scavenging activity of the sulfated polysaccharide from *Ulva sp.* was compared with gallic acid. The reported activity was 68.12 ± 0.44%, as shown in Fig. 6.

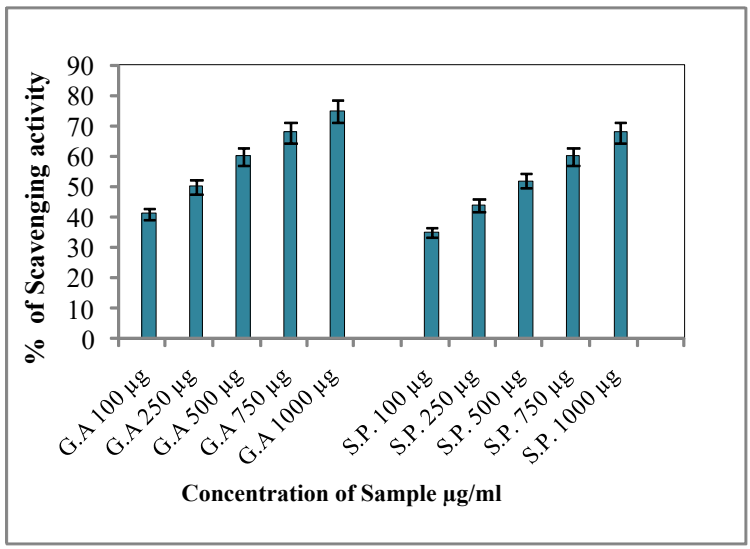


Fig. 6. Hydroxyl scavenging activity of SP

Emulsification index

The emulsification index (E24) of the sulfated polysaccharide as 249 ± 0.5% for olive oil, 217.7 ± 0.5% for sunflower oil, 233 ± 0.5% for groundnut oil, 223.3 ± 0.5% for castor oil, and 209 ± 0.5% for toluene. The obtained values of 250 ± 0.5% for sesame oil, 220 ± 0.5% for kerosene, 247 ± 0.5% for diesel, 246 ± 0.5% for coconut oil, and 255.6 ± 0.5% for palm oil Fig: 7.

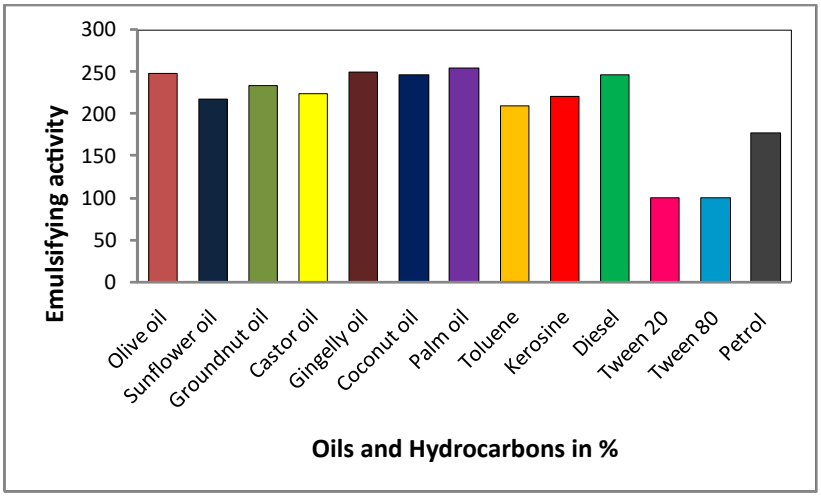


Fig.7. Emulsification index of sulfated polysaccharide extract against various hydrocarbons/ vegetable oil

Discussion

Marine algae are sources of bioactive compounds, including antioxidants and compounds with reported antiviral and anti-inflammatory activities. Some marine algal polysaccharides have also been investigated for anticoagulant properties. These findings support continued research into marine-derived bioactive compounds (Lahaye and Kaffer, 1997; Mahendran et al., 2024; Palpperumal et al., 2025).

Alshaikheid et al. (2019) reported a 7% yield of sulfated polysaccharides from *Stichopus tremulus*. Polysaccharide yield can vary with harvest time and extraction conditions. In the present study, the reported yield after partial purification with DEAE-cellulose was 4.15 g for *Ulva sp.* Palpperumal et al. (2024) reported a 5.69% yield of sulfated polysaccharides from Padina.

The biochemical composition of sulfated polysaccharides from *Ulva sp.* was investigated by estimating carbohydrate, sulfate, and uronic acid contents. Palpperumal et al. (2024) reported glucose (79.54 ± 0.78%), sulfate (17.16 ± 0.69%), and uronic acid (9.49 ± 0.78%) in sulfated polysaccharides from *Padina pavonica*. Na et al. (2010) reported that water-soluble sulfated polysaccharides from *Capsosiphon fulvescens* contained 4.8% uronic acid, 5.7% sulfate, and 49.8% neutral sugars.

Pierre et al. (2011) investigated the antibacterial activity of a sulfated galactan extracted from the marine alga *Chaetomorpha aerea* against *Staphylococcus aureus*. In the present study, the sulfated polysaccharide from *Ulva sp.* was tested against the listed human-associated bacterial pathogens using a diffusion assay. The strongest activity against the clinical isolates was reported for *E. coli,* while the weakest was reported for *Pseudomonas aeruginosa* at the tested concentration. Harinathan et al. (2025) also reported concentration-dependent antimicrobial activity in Kappaphycus sp. required to establish the active compounds and their potential applications.

The results suggest that the *Ulva sp.* sulfated polysaccharide extract has in vitro free-radical-scavenging activity. Harinathan et al. (2025) reported antioxidant activity associated with phycoerythrin. Previous studies have shown that the antioxidant capacity of algal polysaccharides can depend on molecular mass and degree of sulfation (Zhong et al., 2017; Castro et al., 2018; Palpperumal et al., 2024). The reducing-power value reported in the present study was 0.992 ± 0.45.

The hydrogen peroxide-scavenging activity of the sulfated polysaccharide from *Ulva sp.* is reported as 75.32 ± 0.46%. De Souza et al. (2007) reported hydrogen peroxide-scavenging activity in a sulfated fraction from *P. gymnospora*. Harinathan et al. (2025) reported a DPPH-assay IC50 of 0.043 mg/mL, compared with 0.031 mg/mL for ascorbic acid. Previous studies have also examined DPPH radical reduction by antioxidant compounds (Maruthupandi et al., 2014; Fukumoto and Mazza, 2000). Harinathan et al. (2025) reported an ABTS radical-scavenging IC50 of 0.023 mg/mL for phycoerythrin, compared with 0.031 mg/mL for butylated hydroxytoluene (BHT). In the present study, ABTS activity was reported as 64.35 ± 0.66%.

The hydroxyl radical-scavenging activity of the *Ulva sp.* sulfated polysaccharide was reported as 68.12 ± 0.44%. Palpperumal et al. (2024) reported 76.13% hydroxyl radical-scavenging activity for sulfated polysaccharides from *P. pavonica*. Souza et al. (2007) also investigated hydroxyl radical-scavenging activity in a fucoidan fraction from *P. gymnospora*.

The emulsification index (E24) of the *Ulva sp.* sulfated polysaccharide was highest for palm oil (255.6 ± 0.5%) among the values reported. Palpperumal et al. (2024) also described variation in emulsification indices across oils and hydrocarbons. Crude fucoidan from *Saccharina japonica* has been investigated as a surface-active compound with potential industrial applications (Hifney et al., 2026).

Summary and Conclusion

The present study investigated the antioxidant and antimicrobial activities of sulfated polysaccharides extracted from the green alga Ulva sp. collected from the Mandapam coast, Tamil Nadu, India. The extracted polysaccharide was partially purified using DEAE-cellulose chromatography and characterized by estimating its carbohydrate, sulfate, uronic acid, and elemental composition. The results demonstrated in vitro antibacterial activity against the tested bacterial strains, with comparatively higher inhibition reported against *Escherichia coli* than against *Pseudomonas aeruginosa*. The sulfated polysaccharide extract also exhibited free-radical-scavenging activity in different antioxidant assays, including DPPH, ABTS, hydrogen peroxide, and hydroxyl radical-scavenging assays, along with measurable reducing power and total antioxidant capacity.

In addition, the extract showed emulsification activity with various oils and hydrocarbons, indicating potential surface-active properties. Overall, these findings suggest that sulfated polysaccharides from Ulva sp. may serve as promising sources of biologically active marine-derived compounds with potential applications in food, pharmaceutical, and related biotechnological fields. However, further studies involving detailed structural characterization, standardized quantitative assays, toxicity evaluation, and appropriate in vivo investigations are necessary to establish their biological mechanisms, safety, and practical applicability.

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