Original Article

ANTIOXIDANT POTENTIAL AND SOME MEDICINAL PROPERTIES OF AGAR FORMULATIONS FROM SEAWEED *Gracilaria filiforms* IN COASTAL TAMIL NADU

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Abstract

Fractionated polysaccharides were produced by extracting the water soluble polysaccharides (WSP) from the seaweed *Gracilaria filiforms* (FPs). The FP was structurally characterised using FT-IR, and the antioxidant capacities were assessed utilising tests for total antioxidant, 2,2 diphenyl-1-picrylhydrazyl (DPPH), and hydrogen peroxide radical scavenging (H_2O_2). All of the antioxidant experiments revealed increased scavenging activity in FPs from *Gracilaria filiforms.* The FPs from all the seaweed shown admirable anticoagulant properties. The greatest larvicidal activity on *Anopheles stephensi* and *Aedes aegypti* was demonstrated by FPs from *Gracilaria filiforms.* The outcomes clearly demonstrate the FPs from the three Indian seaweeds that were chosen as prospective future functional foods, nutraceutical substances, and insecticides.

Keywords: Seaweeds, *Gracilaria filiforms*, Red algae, FT-IR, Anticoagulant agent, Larvicidal activity.

Introduction

Seaweeds are abundant in bioactive components with nutritional value and a wide range of medicinal characteristics, and they are renewable resources [1, 2]. Comparing marine algae to other marine microbes and animals, scientists have discovered that marine algae are special and appealing prospects in the disciplines of biochemistry and pharmacology [3]. Seaweeds are organisms that resemble plants and are typically split into the following three groups: brown algae, red algae, and green algae. Due to their abundance of bioactive substances, seaweeds are gaining interest in the biomedical and food industries [4,5]. Seaweeds have significant roles in human metabolic processes [8] and include a variety of compositions of critical minerals, trace elements, and other biomolecules required for human nutrition [6, 7]. Brown and red macroalgae's polysaccharides have the potential to be employed as functional foods and natural medicines [8]. The most significant polysaccharides are

those produced by red algae, such as carrageenans and agar, brown algae, such as fucoidans, laminaran, and alginates, and green algae, such as galactans, mannans, and xylans, which are employed in pharmaceutical products on a commercial scale [9]. The biological effects of the polysaccharides, such as their anticoagulant, antioxidant, anticancer, and immunomodulatory actions, are intimately related to these substances [10,11]. Antioxidants have recently gained recognition for their critical functions in the protection of infections and degenerative illnesses. According to several studies, seaweed polysaccharides have potent anti-free radical properties and the unusual ability to show oxidative damage in living things [12]. Antioxidant properties in a variety of marine algae have been reported [7,15]. Anticoagulants are frequently employed in clinical settings as well as in in vitro medical procedures including dialysis and pre- and postoperative operations [16]. Heparin is a drug that is primarily derived from cows and is used extensively in medicine to treat and prevent venous and arterial thrombosis. Heparin has been linked to severe bovine spongiform encephalopathy, and developing nations have also seen reports of adverse effects in humans [17,18]. Brown, red, and green algal polysaccharides are cited in this regard as possible sources for anticoagulant activity [12,19,20]. The most harmful insects are mosquitoes, which can spread diseases including West Nile Fever, yellow fever, dengue, and filariasis that can impact both humans and animals [21,22]. In India and other West Asian nations [23], as well as in tropical nations, *Anopheles stephensi* and *Aedes aegypti* are the main mosquito vectors for malaria. The focus of recent research on seaweed polysaccharides as an alternate source for mosquito vector control has been on developing new techniques to eradicate mosquitoes globally. The present study is focussed on invitro antioxidant potential and anticoagulant property and larvicidal properties of agar formulated from Seaweed *Gracilaria filiforms.*

Materials and methods

Collection of seaweed

Red algae known as *Gracilaria filiforms*, a marine seaweed, were gathered from the coastlines of India's southeast coast. The gathered seaweed samples were rinsed with tap water first, then with distilled water to remove any impurities, sand pieces, adhering salts, and the surrounding biota. A freeze dryer was used to dry the samples. A mixer mill was used to finely powder the dried components, and the powdered samples were then kept in a desiccator until utilized.

Extraction of aqueous polysaccharide

5 g of powder sample of *Gracilaria filiforms* were dissolved in 100 mL of milli Q water and autoclaved at 121 °C for 1 hour. After filtering the samples to get rid of unwanted components, the isolated water soluble polysaccharides were spun at 5000 rpm for 15 minutes. Until gel formation, the solutions were maintained at room temperature. The sample was stored at -80 °C for future usage after being dried at 60 °C for an overnight period.

Fractionation of polysaccharide (FP)

The carbopol 934 was liquefied slowly while stirring in 60 ml of Milli-Q water for 1 hour to evade agglomeration. Carbopol was then neutralized with triethanolamine (5.6 ml) with stirring. This was followed by the addition of methylparaben (0.6 g) and propylparaben (0.07 g) with continuous exhaustive stirring until medium components were homogeneous, then mannitol (17.5 g), lysine (0.88 g), and glycerol (8.75 ml) were added; finally, the gelated agar (100 g) core component of the formulation was added. The mixture was mixed well, and it was stirred for 30 min (Magnetic stirrer, PCE Pvt. Ltd. India) until a clear consistent gel base was obtained. The formulated agar was stored in an air-tight container under freeze conditions.

Structural analysis

Fourier Transform Infrared FT-IR) Spectroscopy

A beta FT-IR spectrophotometer was used to perform Fourier Transform infrared spectroscopy in order to identify the functional groups of the FPs (Bruker). Between 4000 and 500 cm-1, the FPs were examined. A 0.5–1mm thick film containing 1 mg of FPs and KBr was combined, and the results were examined [24].

***Invitro* antioxidant activity of FP**

Total antioxidant assay

The phosphomolybdenum assay technique used by Prieto et al. [25] was used to determine the total antioxidant capacity of the FP. A mixture of 3 mL of reaction mixture solution (0.6 M H2SO4, 28mM sodium phosphate, and 4 M ammonium molybdate) and about 0.5 mL of FP sample were used. After 3 hours of incubation at 95 °C, the reaction mixtures were cooled to room temperature. Ascorbic acid served as the standard reference as the absorbances were measured at 695 nm. The total amount of antioxidant activity was measured as the milligramme equivalents of ascorbic acid per gramme of FP.

2,2 diphenyl-1-picrylhydrazyl (DPPH) assay

The FP were diluted in 2 mL of 0.135 mM DPPH methanolic solution at various doses (25, 50, 100, 150, and 200 µg/ml). The UV-Vis spectrophotometer was used to measure the decrease in OD values at 517 nm following 30 min of incubation at 30 °C in the dark. Ascorbic acid served as the reference standard against the sample were compared. According to the methodology used by Suresh et al. and Gong et al. [26,27], the inhibition percentages were determined using the common formula.

Hydrogen peroxide (H_2O_2) scavenging assay

The Fernando et al. [28] methodology was used to assess the hydrogen peroxide scavenging abilities of FP. With PBS buffer, about 20mM of hydrogen peroxide (H2O2) was made (pH 7.4). A total of 1 mL of FP were prepared at different concentrations (25, 50, 100, 150, and 200 g/ml), and 0.6 mL of PBS-dissolved H2O2 was added. The reaction mixtures were read at 230 nm in a UV-Vis spectrophotometer using ascorbic acid as the standard after 30 minutes of incubation.

Anticoagulant activity

A kit purchased from the Instrumentation Laboratory was used to assess the activated partial thromboplastin time (APTT) and prothrombin time (PT). 100 µl of plasma from healthy donors that had different FP concentrations was incubated at 37 °C for 3 minutes. 100 µl of bovine cephalin and 100 µl of prewarmed 0.25M $CaCl_2$ were added, the mixture was incubated for 3 min, and the clotting times were recorded. The standard was used to compare the observed APTTs, and the activities were reported as IU/mg. In the PT experiment, citrated normal human plasma (90 µl) and the FP solution (10 µl) were combined and incubated for 10 min. A coagulometer was used to measure the clotting time after the addition of 200 µl of PT reagent, which had been preincubated for 10 min at 37 °C, by adopting the methodology of Moura et al., [29].

Larvicidal activity

Larval mortality assay was carried out using larvae of the fourth instar by the methodology of 6071lumalai et al. With 1 mL of DMSO added as the negative control, 25 larvae were added to 249 mL of distilled water. The dead

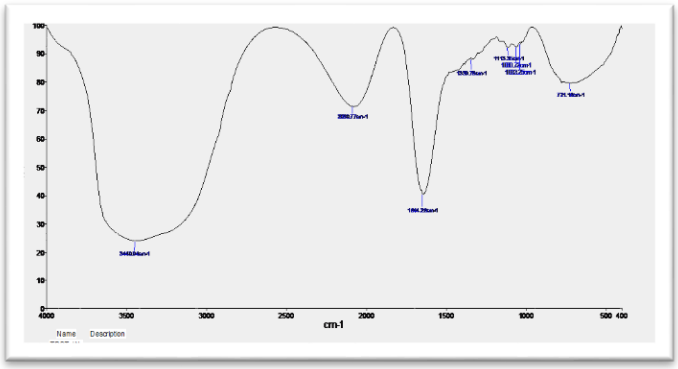
larvae were counted and the proportion of dead larvae for the three replicates was computed after 24 hours of exposure. Abbott's method was used to adjust the percentage of larval mortality. By using a probit analysis, the replicates were used to determine the average LC_{50} values [24,30].

Results and discussion

FT-IR analysis

Gracilaria filiforms, a red algae, produced FPs that showed a band at 3451.29 cm^{-1} (Fig. 1), which corresponded to OeH stretching and indicated the alcohols and phenols. Alkanes were present, as evidenced by the absorbance of 2961 cm^{-1}. Alkenes were blamed for the significant peak at 1642 cm^{-1}. Aliphatic amines were present as indicated by CeN stretching vibrations in the band between 1192.21 and 1100.70 cm^{-1}. According to CeOeC of 3, 6-anhydro-L-galactose vibrations, the peak at 931.95 cm^{-1} was measured [31]. The vibrations of aromatic groups' CeH stretching were attributed to the peak at 889.36 cm^{-1}.

Figure 1: FT-IR spectrum of FPs from seaweed- *Gracilaria filiform*



Invitro antioxidant activity of FP

Table 1: *Invitro* antioxidant potential of FP from seaweed

Concentration µg/ml	DPPH Radical scavenging	Total antioxidant assay	Hydrogen peroxide scavenging activity
25	13.51±0.06	15.47±0.45	18.20±0.05
50	24.79±0.12	28.47±0.25	34.44±0.47
100	36.54±0.23	41.47±0.22	49.12±0.23
150	56.58±0.25	61.45±0.28	66.47±0.23
200	67.47±0.52	70.33±0.99	75.47±0.25
IC_{50}	72.55	64.13	51.43

A wide variety of illnesses and health issues, including cardiovascular, neurological, cancer, and pulmonary issues, have been linked to free radical reactions in the body. Free radicals play a big part in how quickly we age. Antioxidants may be a viable therapy for such abnormalities. Reactive oxygen species such superoxide radicals, hydroxyl radicals, and hydrogen peroxide are produced by a variety of oxidative reactions. The imbalance between

antioxidant enzymes and reactive oxygen species is a serious issue for our health. The ability of plant extracts to scavenge diverse ROS species was investigated using separate assays for each type of reactive oxygen species. The radical 1,1-diphenyl-2-picrylhydrazol is comparatively stable. Antioxidant activity is highest at the lowest IC_{50} value. Therefore, the phenolic components of the test extract may be in charge of removing H_2O_2. The orthodihydroxy phenolic structure of substances like catechin and quercetin shields bacterial cells against cytotoxicity brought on by H_2O_2 [32]. Increasing total antioxidants has the potential to treat neurological conditions and has the power to treat cancer, diabetes, and respiratory disorders [33].

Anticoagulant activity

Table 2: Clotting time of FP extracted from seaweed

Concentration (µg/ml)	**APTT**	**PT**
20	3.57±1.42	1.25±3.68
40	12.0±5.35	2.36±6.58
60	16.7±8.54	6.69±6.39
80	23.3±9.27	10.3±5.68
100	30.6±2.57	13.6±2.87

FP from the red seaweed *Gracilaria filiforms* prolonged the coagulation of human plasma with an APTT of 30.6±2.57 and PT of 13.6±2.87 at 100 µg/ml. The current investigation focused on the initial synthesis of fresh seaweed-derived anticoagulant molecules. These chemicals are frequently formed as secondary metabolites and sulfated polysaccharides, and marine bioactive compounds have frequently been found to have anticoagulant activity [34]. Sulfated polysaccharides from green, brown, and red seaweeds have been addressed as anticoagulants by Wang et al. [35]. In Caulerpa racemosa, sulfated polysaccharides, antiviral and anticoagulant properties have been found [36]. Sulfated galactan and sulfated galactoarabinoglucan from the Codium cylindricum and green algae of the Monostroma genus, as well as sulfated galactoarabinoglucan from Codium pugniformis, were reported to have considerable anticoagulant properties. Based on the experimental investigation, the current study has revealed a significantly prominent activity compared to the past studies on anticoagulant action.

Larvicidal activity

Concentration (µg/ml)	**Larvicidal Activity**	
	Anopheles stephensi	*Aedes aegypti*
250	95.0±2.36	98.3±6.25
125	80.6±3.89	87.0±2.58
62.5	60.7±3.67	65.2±5.36
31.25	54.3±9.62	32.5±2.87
16.25	23.6±3.25	25.5±6.39

The larvicidal activity of FP extracted from *Gracilaria filiforms* was good in both *Anopheles stephensi* and *Aedes aegypti.* Similar research was conducted by Salvador-Neto et al. on the halogenated sesquiterpene (+)-Obtusol, that was obtained from the red algae *Laurencia dendroidea* J. Agardh, and which shown larvicidal efficacy against the dengue vector mosquito Aedes aegypti at an LC_{50} value of 3.5 ppm. Another investigation using crude extracts from the two seaweed, *Dictyota dichotoma* and *Enteromorpha intestinalis,* demonstrated the larvicidal potential against *Aedes aegypti* with LC_{50} values of 0.0683±0.0084 µg/ml and 0.0744±0.0086 µg/ml, respectively, and LC_{90} values of 0.1401 and 0.1399, respectively [36].

Conclusion

In the current study, the chemical contents of the FP from *Gracilaria filiforms* (red algae) were identified together with their structural characteristics using FT-IR spectroscopy. The seaweed's FP demonstrated remarkable antioxidant and radical scavenging abilities. All types of seaweed's FP have also shown strong in vitro anticoagulant effects (APTT, PT), confirming their explicit participation in the extension of the intrinsic coagulation pathway's time. Furthermore, *Anopheles stephensi* and *Aedes aegypti* have been shown to be very susceptible to the FP from the seaweed. However, more research is required to confirm that seaweed holds great promise for the future as functional food ingredients and nutraceutical agents with significant biological value.

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