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Antioxidant and Antibacterial Properties of Methanolic Extract of Green Seaweed *Chaetomorpha linum* From Gulf of Mannar: Southeast Coast of India

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ABSTRACT

Background: Plants are an essential and integral part of complementary and alternative medicine due to their ability to generate secondary metabolites that are used to restore health and treat many diseases.
Objectives: The aim of the present study was to determine the antioxidant and antibacterial activities of the green seaweed *Chaetomorpha linum*.
Materials and Methods: The antioxidant and antimicrobial activities of *C. linum* from the Mandapam coastal region of the Gulf of Mannar, on the southeast coast of India, were examined based on the free radical-scavenging activity of the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), ferrous reducing antioxidant property (FRAP), and total phenolic content in the methanolic extract. The antibacterial properties of the methanolic extract of *C. linum* were tested against pathogenic bacterial strains, including *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Proteus mirabilis, Klebseilla pneumoniae,* and *Salmonella typhimurium,* by cup-plate agar diffusion method.
Results: The DPPH scavenging activity was equivalent to an IC_{50} value of 9.8 μg/mL ascorbic acid. The total phenolic content was 672.3 mg/g gallic acid equivalent, and the IC_{50} value by FRAP assay was 8.2 μg/mL. The *C. linum* extract showed significant activity against the majority of bacteria, comparable with standard antibiotics.
Conclusions: *C. linum* has potential as a natural antioxidant and a natural source of antimicrobials against many microbes.

Implication for health policy/practice/research/medical education:
The *C. linum* may be used as an effective antioxidant and antimicrobial agents to combat various ailments caused by the free radicals and the microbial species.

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1. Background

In biomedicine, much attention has been paid to natural antioxidants and their association with health benefits (1). Many studies have on the biological activities of seaweed have reported it to be a potential source of natural antioxidants (2, 3). Among the features of marine algae and its components, several extracts have been screened with regard to antioxidant and radical scavenging activity, using stable free radicals (4-7).

Traditionally, seaweeds have been used in the treatment of various infectious diseases. Many substances obtained from seaweeds have been used for decades in medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties (8-11). Furthermore, several groups have investigated the properties of green, red, and brown seaweed (12, 13).

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Chaetomorpha linum, a green seaweed that is widespread in the Mandapam coastal region of the Gulf of Mannar on southeast coast of India. (Class: *Ulvophyaceae*; Order: *Cladoporales*; Family: *Cladoporaceae*), is mainly used for food, animal feed, and agriculture.

The majority of seaweeds from the Gulf of Mannar have not been examined for their bioactive substances, and until now, no screen of antioxidant activities has been performed with *C. linum*,despite the abundance and diversity of algae in coastal waters (Southeast coast of India).

2. Objectives

In this investigation, we evaluated the antioxidant and antibacterial activity of a methanolic extract of *C. linum*, obtained from the Gulf of Mannar, a southeastern coastal region of India.

3. Materials and Methods

3.1. Chemicals

DPPH (2,2 diphenyl – 1 picryldrazylhydrate) and ascorbic acid were purchased from Hi media (Mumbai, India), and potassium ferricyanide was obtained from Merck (Mumbai, India). All other chemicals were obtained commercially and were analytical-grade.

3.2. Sample collection

In the present study, *C. linum* (Muller) Kutzing green seaweed was collected from the Mandapam coastal region (78 °8'E, 9 °17'N), in the Gulf of Mannar, Tamilnadu, South India, on low tide in December 2009, brought immediately to the laboratory in polythene bags, and washed several times with seawater to remove sand, mud, and attached fauna. The algae was cleaned using a brush to remove epiphytes with distilled water. After cleaning, the algae was dried in the shade at room temperature for 1 week. The dried algal materials were homogenized to a fine powder and subjected to extraction.

3.3. Preparation of Extracts

Five hundred grams of powdered *C. linum* seaweed sample was taken and extracted successively with methanol (90%) using a soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to obtain their corresponding residues. The methanolic extracts were further subjected to antioxidant and antibacterial assays in triplicate.

3.4. DPPH Radical Scavenging Assay

The radical-scavenging activity of methanolic *C. linum* extracts against DPPH radicals was determined by the method of Blois *et al* (14). DPPH (0.1 mM in methanol) was prepared, and 1.0 mL of this solution was added to 3.0 mL of extract in methanol at various concentrations (1-16 μg/mL). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without extract. Ascorbic acid at various concentrations (1 to 16 μg/mL) was used as the standard. A lower absorbance of the reaction mixture indicates greater free radical-scavenging activity. The ability to scavenge DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = A_{control} - A_{test} / A_{control} \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the extracts. The antioxidant activity of the *C. linum* extract was expressed as IC_{50} and compared with the standard. The IC_{50} value was defined as the concentration (in μg/mL) of extract that inhibited the formation of DPPH radicals by reducing power assay.

The reducing power of methanolic extracts of *C. linum* was determined (15). Various concentrations of the extracts (1-16 μg/mL) in 1.0 mL of deionized water were mixed with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL). The mixture was incubated at 50°C for 20 min, and aliquots of trichloroacetic acid (2.5 mL) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and freshly prepared ferric chloride solution (0.5 mL). The absorbance was measured at 700 nm. A blank was prepared without extract. Ascorbic acid at various concentrations (1 to16 μg/mL) was used as the standard. Increased absorbance of the mixture indicates an increase in reducing power.

$$\text{\% Increase in Reducing Power} = A_{test} / A_{blank} - 1 \times 100$$

where A_{test} is the absorbance of the test solution and A_{blank} is the absorbance of the blank. The antioxidant activity of the seaweed extract was expressed as IC_{50} and compared with the standard.

3.5. Determination of Total Phenolic Content

Total phenolic content of the *C. linum* extracts was determined using the Folin-ciocalteu reagent (16). One milliliter of extract in Gallic acid (20, 40, 60, 80, and 100 mg/L) was added to a 25-mL volumetric flask, containing distilled deionized water. The blank reagent was distilled deionized water. One milliliter of Folin-ciocalteu phenol reagent was added to the mixture and mixed by shaking. After 5 min, 10 mL of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to 25 mL with deionized distilled water and mixed. After incubation for 90 min at room temperature, the absorbance against the prepared blank reagent was measured at 750 nm on a spectrophotometer. Total phenolic content of the seaweed was expressed as mg Gallic acid equivalents (GAEs) or 100 g fresh weight. All samples were analyzed in triplicate.

3.6. Antibacterial Activity

The following strains of bacteria were used: *Staphylococcus aureus* (MTCC No. 96), *Bacillus cereus* (MTCC No. 430), *Escherichia coli* (MTCC No. 443) *Proteus mirabilis* (MTCC No. 425), *Klebseilla pneumoniae* (MTCC No. 432), and *Sal-*

monella typhimurium (MTCC No. 98) were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures were maintained on nutrient agar (Hi Media, India) slants at 4°C and were subcultured before use.

The antibacterial activity of *C. linum* was studied by cup-plate agar diffusion method (17). The turbidity of each bacterial suspension was adjusted the optical density a 0.5 McFarland standard, resulting in a suspension containing 1.5 x 10^8 CFU/mL. Mueller- Hinton agar was prepared, inoculated with bacterial cultures, and transferred to sterile 15-cm diameter Petri dishes. The medium in the plate was allowed to set at room temperature for 10 minutes and solidify for 30 minutes. Three wells (6-mm inner diameter) were made in each plate. Stock solutions of the test residual extract were prepared at concentrations of 100, 300, and 500 mg/mL. One hundred microliters of each concentration was placed in the well with sterile pipettes. In each plate, 1 well was used for the control, standard, and test respectively. Chloramphenicol (100 µg/mL) was used as the standard, and the respective solvent was used as the control. The petri dishes were incubated for 16 h at 37°C and examined with regard to size of the zones of inhibition. Methanol was used as the control. The length of the inhibition zone was measured in millimeters from the edge of the well to the edge of the inhibition zone, and the results were tabulated.

4. Results

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The comparison of the antioxidant activity of the extracts (at 1, 2, 4, 8, and 16 µg/mL) and reference standard is shown in *Table 1*. The methanolic extract of *C. linum* exhibited a significant dose-dependent inhibition of DPPH activity, with an IC_{50} value of 9.8 µg/mL. The IC_{50} value of the extract was comparable with that of the reference standard, ascorbic acid (IC_{50} = 5.8 µg/mL), indicating the antioxidant activity of *C. linum*. The IC_{50} value in the reducing power assay was 8.2 µg/mL and 3.2 µg/mL, respectively, for the methanolic extract of *C. linum* and ascorbic acid. By Folin-Ciocalteu method, the highest total phenolic content of *C. linum* was 672.3 mg/GAE/100 g/extract.

The methanolic extract of *C. linum* was tested for its antibacterial activity by cup-plate agar diffusion method. The results of the antibacterial studies with regard to zone of inhibition are shown in *Table 2*. The methanolic extract of *C. linum* exhibited activity against most of the tested strains, showing the highest activity against *B. cereus* and *P. mirabilis* (500 µg/mL) and the lowest activity against *E. coli* (300 µg/mL) ; no activity was observed against *K. pneumoniae*.

5. Discussion

The identification of antioxidants from medicinal plants is a fast-growing field of research, and many antioxidants have been investigated by several methods. The DPPH assay is a quick, reliable, and low-cost method that has frequently been used to evaluate the antioxidative potential of various natural compounds (18).

Methanolic extracts of *C. linum* exhibited potent antioxidant activity in a dose-dependent manner, by DPPH radical

Table 1. Effect of Methanolic Extract of *Chaetomorpha linum* on DPPH Radical Scavenging and Reducing Power Activity

Concentration, µg/mL [a]	DPPH [b] Radical Scavenging Activity, %		Reducing Power Activity, %	
	Chaetomorpha linum	Standard	*Chaetomorpha linum*	Standard
1	6.4 ± 0.002	16.98 ± 0.002	16.62 ± 0.005	24.04 ± 0.002
2	14.57 ± 0.001	28.42 ± 0.002	25.82 ± 0.006	40.05 ± 0.004
4	28.74 ± 0.003	42.55 ± 0.007	33.78 ± 0.001	58.58 ± 0.003
8	45.32 ± 0.001	65.78 ± 0.003	49.17 ± 0.006	77.39 ± 0.007
16	75.09 ± 0.002	92.91 ± 0.002	73.44 ± 0.001	83.41 l ± 0.001
IC_{50}	9.8 µg/mL	5.8 µg/mL	8.2µg/mL	3.2 µg/mL

[a] Each value is expressed as Mean ± Standard Deviation (n = 3)
[b] Abbreviations: DPPH, 2, 2 disphenyl-1 picryldra zylhydrate

Table 2. Antimicrobial Activity of the Methanolic Extract of *C. linnum*

Concentration, µg/mL [a]	Gram Positive Organism		Gram Negative Organism			
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Salmonella typhimurium*	*Proteus mirabilis*	*Klebsiella Pneumoniae*
100	11	21	-	15	22	-
300	13	23	03	16	24	-
500	14	27	05	17	25	-
Standard 100	23	23	24	25	23	22
Control	-	-	-	-	1	-

[a] Each Value is Expressed As Average (n = 3): No Inhibition.

quenching assay. The methanolic extract of *C. linum* contains a high amount of phenolic compounds, exhibited the greatest antioxidant activity (as ascorbic acid equivalents) in this study. The higher scavenging activity of *C. linum* may be attributed to hydroxyl groups in the phenolic compounds, which might provide the essential component.

Reducing power is associated with antioxidant activity In this study, the reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) was measured in the methanolic extract of *C. linum*. All tested concentrations of methanolic extract showed significant activity when compared with the standard, ascorbic acid. The methanolic extract of *C. linum* exhibited a concentration-dependent increase in reducing power. Phenolic compounds are major contributors to the antioxidant capacity of plants; function in plant defense mechanisms that counteract reactive oxygen species (ROS) to survive; and prevent molecular damage and damage by microorganisms, insects, and herbivores (19, 20). These antioxidants also possess diverse biological activities, such as anti-inflammatory and anticarcinogenic actions (21).

In this study, the maximum total phenolic content *C. linum* of was 672.3 mg/GAE/100 g/extract. Many bioactive and pharmacologically active substances have been isolated from seaweeds and reported to exhibit antibacterial activity (22). The methanolic extract of *C. linum* showed strong antimicrobial activity against selected human pathogens and has considerable antimicrobial acitvity against a number of microorganisms. The largest zone of inhibition was observed against *Bacillus cereus* and *Proteus mirabilis*. These results are notable, because they were obtained with methanolic extracts, which are not pure products but have good potency. Our results should prompt further studies to isolate and identify the active compounds and evaluate a possible synergism between components with regard to their antioxidant and antimicrobial activity.

Considering the total phenolic content, reducing power, and DPPH radical scavenging activity as indices of antioxidant activity of the extract, our findings demonstrate its potential as a source of natural antioxidants, indicating that *C. linum* is a promising agent in scavenging free radicals and treating diseases that are related to free radical reactions. This work provides insight into the molecular basis of the therapeutic properties of *C. linum* in traditional medicine. The antibacterial study revealed that the methanolic extract of *C. linum* contains certain constituents with significant antibacterial properties. Detailed studies on the isolation and characterization of the plant extract as well as *in vivo* assays will be necessary to discover new biological antioxidants and antibiotics.

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