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RESEARCH ARTICLE

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PHYTOCHEMICAL CHARACTERIZATION AND ANTIMICROBIAL EFFICIENCY OF SEAWEED SAMPLES, *Ulva fasciata* and *Chaetomorpha antennina.*

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ABSTRACT

The seaweeds are a promising source of natural products. In the present study, preliminary phytochemical analysis concluded that due to the presence of extra phytochemical in *Ulva fasciata* when compared to the *Chaetomorpha*, this alga is a beneficial one for its biological activity. In the DPPH scavenging assay, both the seaweed extracts showed high antioxidant activity. The *Ulva fasciata* samples have more effective antioxidant activity when compared to the *Chaetomorpha antennina*. And the percentage of scavenging was found to be about 83.95% *for U. fasciata* and 63.77% for *C. antennina* sample. The rapid TLC assay is considered as the rapid test to evaluate the antioxidant activity of natural compounds. The compounds showing the bands at hR_f = >10, 25 and 94 of both the seaweed extracts and hR_f = 52 in *Ulva sp* alone were proved to be having antioxidant activity. The results of antimicrobial activity by the well diffusion assay also clearly expressed that *Ulva fasciata* has high concentration of active principles when compared to the *Chaetomorpha antennina*.

INTRODUCTION

Seaweeds are one of the important marine living resources and are excellent source of Vitamins (A, B, B12, C, D& E), riboflavin, niacin, panthothanic acid and folic acid as well as minerals such as Ca, P, Na and K. Seaweed dietary fibers perform varied range of functions such as antioxidant, antimutagenic, anticoagulant, antitumor etc., (Dhargalkar *et al.*, 2005). In nature there are a large number of different types of antimicrobial compounds that play an important role in the natural defense of all kinds of living organisms (Ilhami Gulcin *et al.*, 2003).

Marine organisms are emerging as good candidates as an alternate source for bioactive substances. Che (2004) surveyed the occurrence of organic compounds from marine organisms that have been reported to possess antiviral activities. Metabolites from micro-organisms is a rapidly growing field due atleast in part to the suspicion that a number of metabolite obtained from algae and invertebrates may be produced by associated microbes. Studies are concerned with bacteria and fungi isolated from seawater, sediments, algae, fish and mainly from invertebrates such as sponges, molluscs, tunicates, coelenterates and crustaceans. Several researchers have made attempts to identify organisms producing bioactive substances and met with success (Pietra 1997 and Kelecom 2002). There are reports that seaweeds are also rich source of antioxidant compounds (Elena *et al.*, 2001 and Kuda *et al.*, 2005). Hence in the present study is concerned screening of phytochemicals, antioxidants and antimicrobial properties of *Ulva fasciata* and *Chaetomorpha antennina.*

MATERIALS AND METHODS

Extraction of algal sample: The seaweed samples, *Ulva fasciata* and *Chaetomorpha antennina* were collected from Vizhinjam seashore of Kerala. The sample was dried in the shadow; it was powdered and stored at room temperature. About 15g of the algae were extracted with 150 ml of ethanol for 1 week at room temperature. These samples were filtered using Whatmann filter paper and the filtrate was evaporated to dryness under vacuum at 40^0C. Each concentrated extracts were made into different concentrations (200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml) using ethanol.

***Preliminary Phytochemical Analysis*:** The preliminary phytochemicals from the algal sample extracts were determined. In the preliminary phytochemical analysis of crude extracts of *Ulva fasciata* and *Chaetomorpha antennina* for screened the presence of glycosides, tannin, terpenoids, phenolics, sugar, reducing sugar, saponins, amino acid, Sterol, alkaloid, phenolics and flavanoids were carried out by Hanaa *et al.,* (2008) method.

Antioxidant Assay: The antioxidant properties of the seaweed extracts were studied by their ability to scavenge free radicals using the 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) reducing power.

Preparation of Test Extracts: 5ml of hydroponics test sample was dissolved in 5ml of pure ethanol for analysis.

Preparation of DPPH (2, 2-Diphenyl-1-picryl hydrazyl): 0.0025g of 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) was dissolved in 25 ml of methanol (0.25mM concentration). The content should be made and kept in dark condition, because DPPH is light sensitive.

DPPH free Radical Scavenging Assay: The free radical scavenging activity of seaweed extract was measured by the DPPH method proposed by Hatano *et al.*, 1988. Percentage inhibition or DPPH scavenging activity was calculated by following expressions.

Percentage of scavenging = $[(A_o - A_1)/A_o] \times 100$

Where A_o is the absorbance of control and A_1 is the absorbance of test sample.

Rapid screening with TLC method: Thin Layer Chromatography was used to detect antioxidant activity of two seaweed samples based on spraying the plates with oxidizing reagents. The separated compounds on TLC plates were spraying with 0.004% DPPH stable radical in methanol (Jaime *et al.*, 2005) to located and detect antioxidant active compounds. The protecting against the scavenging DPPH radical gave pale yellow coloured spots were taken as positive results.

Antimicrobial Activity: Ten pathogens were chosen for the present investigation and obtained from Bose Clinical Laboratory, Tamil Nadu, India. Thus, the antimicrobial activity of the crude extract of *Ulva fasciata* and *Chaetomorpha antennina* were determined by measuring the zone of inhibition in the Agar well diffusion method. The results were compared with a standard antibiotic, tetracycline (20 µg/ml).

RESULTS AND DISCUSSION

***Phytochemical analysis*:** In the preliminary phytochemical analysis of crude extracts of *Ulva fasciata* and *Chaetomorpha antennina* contains glycosides, tannin, terpenoids and phenolics. In *Ulva fasciata* extract obtained sterol, alkaloid, phenolics and flavanoids, but absence in *Chaetomorpha antennina.* Sugar, reducing sugar, saponins and amino acid were absent in *Ulva fasciata* extract, but present in *Chaetomorpha antennina* (Table 1).

Table1

Peliminary phytochemical screening of crude extract of Ulva fasciata and Chaetomorpha antennina.

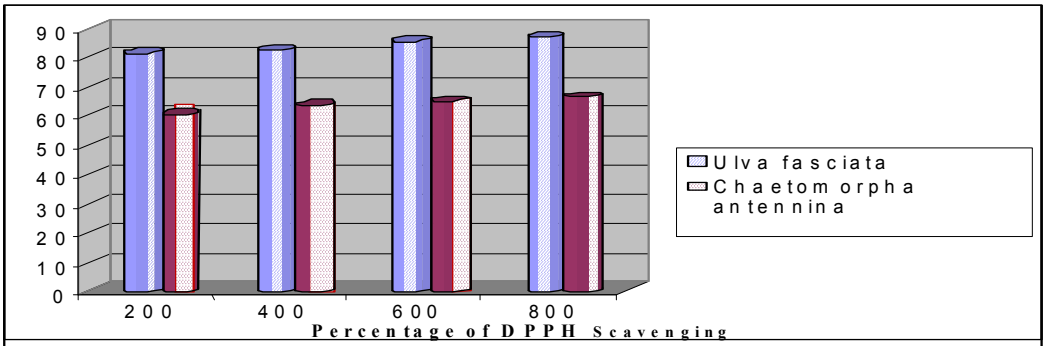
Sl.No	Name of the test	*Ulva fasciata*	*Chaetomorpha antennina*
1.	Alkaloid	Present	Absent
2.	Flavanoid	Present	Absent
3.	Phenolics	Present	Present
4.	Tannins	Present	Present
5.	Saponins	Absent	Absent
6.	Sterols	Present	Absent
7.	Sugars	Absent	Absent
8.	Glycosides	Present	Present
9.	Terpenoids	Present	Present
10.	Reducing sugar	Absent	Absent
11.	Aminoacid	Absent	Absent

Antioxidant activity

DPPH scavenging assay: In the present study the seaweed extracts has high DPPH scavenging capacity, which increased with increasing concentration (Fig. 1). The DPPH assay was carried out at different

concentrations of algal samples, namely 200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml. DPPH assay did not show any significant difference at 200µg/ml and 400µg/ml concentrations in *Ulva fasciata* sample; however, it was significant for 600µg/ml and 800µg/ml for the extracts.

Fig. 1
Effect of crude extract of seaweed samples on scavenging of DPPH



DPPH is a relatively stable free radical. DPPH radical react with suitable reducing agents, the electrons become paired off, and the solution losses colour stoichiometrically depending on the number of electrons taken up. Hence this assay provided information on reactivity of test samples with a stable free radical. The decrease in the absorbance of the DPPH radical caused by test samples was due to the scavenging of radical by electron donation.

Rapid screening with TLC method: Rapid TLC- screening assay based on decolorization of ethanolic DPPH$^{\cdot}$ radical that sprayed into TLC plates, as a rapid test to evaluate the antioxidant activity of natural compounds (Molyneux, 2004). The development of pale yellow spots on the separated TLC plate confirms the antioxidant activity of the two samples. Among all separated bands, bands at hR_f = >10, 25, 52 and 94 of *Ulva fasciata* and *Chaetomorpha antennina* extracts showed an excellent antioxidant activity (Table 2).

Table 2
TLC profile of Ulva fasciata and Chaetomorpha antennina

No. of spots obtained	*Ulva fasciata*		*Chaetomorpha antennina*	
	R_f value	hR_f value	R_f value	hR_f value
1	0.24	24	0.24	24
2	0.52	52	0.78	78
3	0.78	78	0.94	94
4	0.94	94	0	0

***Antimicrobial activity*:** The antimicrobial activity of crude extracts of *Ulva fasciata* and *Chaetomorpha antennina* against ten human pathogenic bacterial strains were done and their zone of inhibition compared with standard antibiotic, tetracycline. The seaweed extracts

were shown more active antimicrobial proficiency against *Pseudomonas aeroginosa*, *Staphylococcus aureus, Escherichia coli*, *Enterococcus aeroginosa* and *Salmonella paratyphi* when compared to the standard antibiotic, but little antibacterial activity against *Klebsiella pneumoniae, Bacillus subtilis, Citrobacter sp.*and *Proteus sp. Staphylococcus epidemis* is an highly resistant against both test samples as well as standard antibiotics. (Table. 3).

Table 3
Antimicrobial activity of the crude extract of U. fasciata and C. antennina against human pathogenic bacteria.

Sl.no.	Pathogenic bacteria	Zone of inhibition (mm)		
		Ulva fasciata	*Chaetomorpha antennina*	Standaard antibiotic Tetracycline
1.	*Staphylococcus aureus*	23	28	30
2.	*Escherichia coli*	25	23	25
3.	*Pseudomonas aeruginosa*	15	20	22
4.	*Bacillus subtilis*	14	15	23
5.	*Enterobacter aeruginosa*	15	7	19
6.	*Citrobacter sp.*	16	18	30
7.	*Staphylococcus epidemis*	-	-	15
8.	*Proteus sp.*	6	6	8
9.	*Salmonella paratyphi*	25	29	30
10.	*Klebsiella pneumoniae*	9	8	21

The seaweeds are a promising source of natural products. From the present study, preliminary phytochemical analysis concluded that due to the presence of extra phytochemical in test extracts, they are beneficial one for its biological activity. In the DPPH scavenging assay, the seaweed extracts showed high antioxidant activity. The test extract samples have more effective antioxidant activity, the percentage of scavenging was found to be about 83.95% *for U. fasciata* and 63.77 % in *Chaetomorpha antennina*. The rapid TLC assay is considered as the rapid test to evaluate the antioxidant activity of natural compounds. The compounds showing the bands at hR_f = >10, 25 and 94 of both the seaweed extracts and hR_f = 52 in *Ulva sp* alone were proved to be having antioxidant activity. Those bands that have developed into yellow spots were suspected as carotenoids and other phenolic compounds with respect to references (Hanaa *et al*., 2008). The results of antimicrobial activity by the well diffusion assay also clearly expressed that test extracts have high concentration of active principles.

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