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Preliminary Screening, Antioxidant and Antimicrobial potential of *chaetomorpha antennina* and *Caulerapa scalpelliformis* invitro study

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ABSTRACT

The seaweeds are a promising source of natural products .From the preliminary phyto chemical analysis we can conclude that the presence of phytochemical in *chaetomorpha antennina* was more significant when compared to the *Caulerapa scalpelliformis.* The present study was conducted for the free radical scavenging potentials by using DPPH radical and also for the s antimicrobial properties of chaetomorpha *antennina* and *Caulerapa scalpelliformis* in methanolic extract. The tested extract exhibited a dose-dependent free radical scavenging action against DPPH radical and significant antimicrobial potential was observed in *Caulerapa* scalpelliformis. From the overall results we can conclude that this seaweed could be used against several diseases and in the food processing industry to preserve foods.

Key words: phytochemical, antioxidant, antimicrobial; free radical, seaweeds.

1. Introduction

Some of them are phytohormones (absicines, auxins, cytokines, gibberellins, ethylene) and microbial enhancers. Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (lindequist & schweder, 2001; Newman *et al.,* 2003). Marine bacteria often produce anticancer and antibacterial substances as a means of maintaining relationships between epiphytic micro environments, inhibiting competing organisms and microbial pathogens (Avendaño-Herrera *et al*. 2005). Many of these secondary metabolites are halogenated, reflecting the availability of chloride and bromide ions in seawater. Interestingly, bromide is more frequently used by algae for organohalogen production, although chlorine occurs in higher concentrations than bromine in seawater. Marine halogenated compounds comprise a varied assembly of compounds, ranging from peptides, polyketides, indoles, terpenes, acetogenins and phenols to volatile halogenated hydrocarbons (Butler et al .,2009). The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers, hydrogen-donating com-pounds, single oxygen quenchers and metal ion chelators (Okawa*et al.,* 2001).Hence, the present study is concerned for the screening of phytochemicals ,antioxidants and antimicrobial potential of *chaetomorpha antennina* and *caulerapa scalpelliformis.*

2. Materials and methods

2.1 Sample collection

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Seaweeds were collected at a depth of 1-2 m from the coastal area of kanyakumari district was donated by Biiogenic laboratories, Namakkal dist, Salem Tamilnadu, India. Algae samples were cleaned of epiphytes and necrotic parts were removed. Then the cleaned samples were rinsed with sterile water to remove any associated debris. Half of these cleaned fresh materials were air-dried as described by (Gonz.lez del Val *et al.,* 2001). The samples were identified as chaetomorpha *antennina* and *caulerapa scalpelliformis*

2.2 Solvent extraction

The samples were shade dried for 15 days and then pulverized into fine powder using pestle and mortar. The extraction was done by Soxhlet extraction techniques. Different solvents were used successively with gradient polarity (aqueous, methanol and ethanol). The extracts were evaporated to complete dryness by vacuum distillation and stored in refrigerator for further use (Akinyemi *et al.,* 2000; Mohanta *et al.,* 2007; Patra *et al.,* 2008).Among the three solvents used for extraction, methanolic extract was used for further analysis in the present study.

2.3 Preliminary phytochemical analysis

The preliminary phytochemical analysis (*chaetomorpha antennina* and *caulerapa scalpelliformis)* was performed for the presence of alkaloids, tannins, steroids, flavonoids, saponins, terpenoids, glucosides, anthraquinones, glycoside, cardio active glycosides according to sadasivam manickam, 1996.

2.4 Antioxidant assay (DPPH free radical scavenging activity of methanolic extract)

The antioxidant properties of the *chaetomorpha antennina* and *caulerapa scalpelliformis* in methanolic extract were studied by their ability to scavenge free radicals using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reducing power according to (Braca A *et al.,* 2002).

The antioxidant activity of *chaetomorpha antennina* and *caulerapa scalpelliformis* in methanolic extract was performed and the same was done for the blank and standard (ascorbic acid) also. The working solutions of the test extracts were prepared by using chreeeeeek methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002g of DPPH was prepared in 100ml methanol and 1 ml of this solution was mixed with 0.1ml of extract and kept in dark for 30 min and its optical density was measured at 517 nm using Spectrophotometer. The optical density was recorded and % inhibition was calculated using the formula given below (Bors W *et al.,* 1992).

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A - B \times 100}{A}$$

Where A = optical density of the blank and B = optical density of the sample.

2.5 Antimicrobial activity

Several bacteria (10) choosen for the present investigation was obtained from Biiogenic laboratories, Namakkal dist, Salem Tamilnadu,India. The antimicrobial activity of the

methanolic extract of *chaetomorpha antennina* and *caulerapa scalpelliformis* were determined by measuring the zone of inhibition in the agar well diffusion method .The results were compared with standard antibiotic, streptomycin (20µg/ml)

2.6 Statistical analysis

Each data point was obtained by marking at least 3 independent measurements. The results were expressed as mean ±SD and levels of significance were assessed using ANOVA test and the coefficient of variance and the critical differentiation value were obtained (table 2)

3. Results and Discussion

3.1Phytochemical analysis

Phytochemicals such as Tannins, Saponins, Flavonoids, Steroids, Glycosides, alkaloids, anthraquinones glycosides, cardioactive glycosides of the methanolic extract of the two samples showed significant results. But among the two samples cardioactive glycosides & anthraquinone glycosides was found to be absent in *Caulerapa scalpelliformis* where as *chaetomorpha antennina* showed absence of anthraquinones glycosides alone (Table 1).

Table 1: Preliminary phytochemical screening of methanolic extract of *Chaetomorpha antennina* and *caulerapa scalpelliformis*

S No.	**Phytochemical**	***chaetomorpha antennina***	***Caulerapa scalpelliformis***
		Methanol extract	**Methanol extract**
1.	Tannins	+++	+++
2.	Saponins	+++	+++
3.	Flavonoids	+++	+++
4.	Steroids	+++	++
5.	Glycosides	+	+
6.	Alkaloids	+	+
7.	Anthraquinones glycosides	-	-
8.	Cardioactive Glycosides	+++	-

3.2 Antimicrobial activity

The extracts of two samples of methanol extraction possessed antibacterial activity. The antimicrobial activity was assessed against the clinical pathogen, (*Vibrio alginolyticus, K.pneumoniae, E.coli, s.faecalis, Bacillus coagulans, Staphylococcus auereus, Bacillus subtilis, Proteus vulgaris, Cornybacterium diphtheria, Lactobacillus).* The greater results (Zone of inhibition) was shown in the methanol extract (100 µl) against at ten clinical pathogen , the highest zone of inhibition was measured in the methanolic extract of *Caulerapa scalpelliformis* which inhibit the *Bacillus subtilis* (14 mm) and *E.coli* (12 mm). The *s.faecalis*, was resistant pathogen and it shows the minimum zone of inhibition, Among

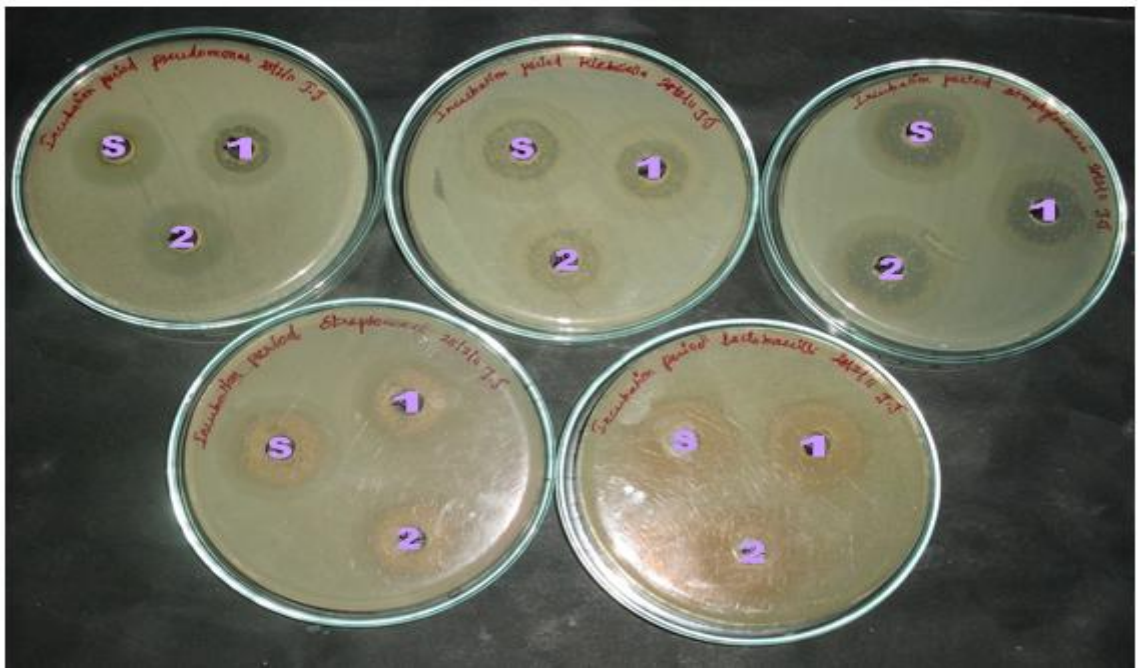
the two samples the *Caulerapa scalpelliformis* shows the greater results when compared to the other sample *chaetomorpha antennina* and also the clinical pathogens were more sensitive to the methanolic extract of the *Caulerapa scalpelliformis* show in the (table 2and plate 1,2).

In this study, some of the pathogenic bacterial strains did not respond to the organic extracts whereas other strains showed some activity. Such unusual response could be attributed to masking of antibacterial activity by the presence of some inhibitory compounds in the extract as observed by (Sastry and Rao, 1994). Some studies concerning the effectiveness of extraction methods light that methanol extraction yields higher antimicrobial activity than n-hexane and ethyl acetate (Rosell and Srivastava, 1987; Moreau *et al.*, 1988; Sastry and Rao, 1994). It is clear that extraction by organic solvents always provide a higher efficiency for antimicrobial activities as compared to water extracts (Masuda *et al.*, 1997; Lima-Filho *et al.*, 2002). The experimental study revealed that methanol aqueous and ethanol extracts caused bigger clear zones than methanol extracts.

Results from the present study have given scientific basis for such traditional medicine practices. In addition, the relative efficiency of seaweeds to inhibit the growth of oral pathogenes has been presented in the present report. Higher plants, as sources of medicinal compounds continue to play dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker, et al., 1995; Cordell, 1995). Data from the present study have implicated as a potential *chactomorpha antenninna* and *Caulerapa scalpelliformis* in such application. And it is hoped that this discovery would be utilized to better the oral health.

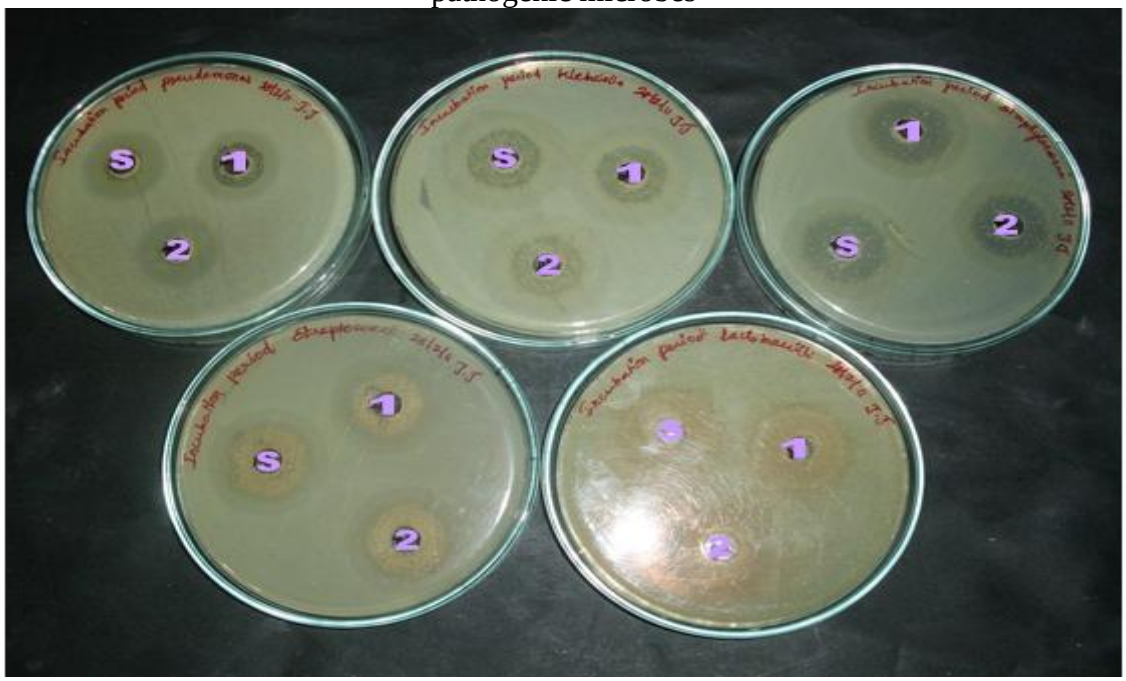
Table 2: Antimicrobial activity of the methanolic extract of *chactomorpha antenninna* and *Caulerapa scalpelliformis*

S. No	***Microorganisms***	***Standard antibiotic streptomycin***	**Methanol extract**	
			Chaetomorpha antennina(zone of inhibition mm)	***Caulerapa scalpelliformis (zone of inhibition***
1.	Vibrio alginolyticus	13	9	8
2.	Klebsiella	12	10	9
3.	Escherichia coli	15	11	12
4.	*streptococcus*.faecalis	10	6	7
5.	Shigella flexneri	20	10	12
6.	Shigellabaydii	8	6	4
7.	Bacillus subtilis	22	11	14
8.	Lactobacillus	15	8	10
9.	Corny bacterium	9	6	12
10.	Shigellasonni	11	8	6



1-Sample (*Chaetomorpha antennina)*
2-Sample (*Caulerapa scalpelliformis)*
S-standard (*streptomycin*)

Plate No 1: Screening of *Chaetomorpha antennina and Caulerapa scalpelliformis against* pathogenic microbes



1-Sample (*Chaetomorpha antennina)*
2-Sample (*Caulerapa scalpelliformis)*
s-standard(*streptomycin*)

Plate no 2: Screening of *Chaetomorpha antennina,* and *Caulerapa scalpelliformis by pouerplate method* against Shigellabaydii, Bacillus subtilis, Lactobacillus, Corny bacterium diphtheria, Shigellasonni.

3.3 Antioxidant activity

The antioxidant activity of the *Caulerapa scalpelliformis* (11.28±0.01 to 21.34±0.05) mg/ml. shows the maximum results of 21.34±0.05 in the methanolic extract while other sample shows the maximum results but *chactomorpha antenninna* lesser than the methanolic extract which shown in the (table 3 and fig 1).

DPPH has been used extensively as a free radical to evaluate reducing substances (Cotelle *et al.,* 1996) and is a useful reagent for investigating the free radical scaveng DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds (Duan *et al.,* 2006). Total methanol extract from *Caulerapa scalpelliformis (*11.28±0.01 to 21.34±0.05) mg/ml .showed significantly higher scavenging activity (Table 3).

In the present study the seaweed extracts has high DPPH scavenging capacity, which increased with increasing concentration (Fig. 8). The DPPH assay was carried out at different concentrations of algal samples, namely 100µg/ml, 200Cg/ml, 300µg/ml. DPPH assay did not show any significant difference at 100µg/ml a 300µg/ml concentrations in *Caulerapa scalpelliformis* sample; however, it was significant for 300µg/ml and for the extracts. 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. The result of our study is supported by (premalatha, 2011).

Table 3: Inhibition % of DPPH radical scavenging activity of standard ascorbic acid and *Chaetomorpha antennina, Caulerap scalpelliformis*

Algal (seaweeds)	**Percentage of inhibition (DPPH radical scavenging activity)**		
Standard (ascorbic acid)	15.75± 0.0.01	18.23 ± 0.02	20.43 ± 0.02
Chaetomorpha antennina	10.53± 0.02	14.34± 0.03	17.32±0.04
Caulerapa scalpelliformis	11.28±0.01	16.30±0.04	21.34±0.05

Values are mean ± SD

The methanol extracts of seaweeds *Caulerpa* scalpelliformis. Showed strong antioxidant activity. In addition, this extract possessed noticeable antimicrobial activity against gram positive and gram-negative bacteria when compared with standard streptomycin. It is evident from the present study that the methanolic extract of Caulerpa scalpelliformis. could beutilized as a good natural source of antioxidants and a possible food supplement or as an

antimicrobial agent in pharmaceutical industry. However, the active components responsible for the antioxidant and antimicrobial activities need to be evaluated.

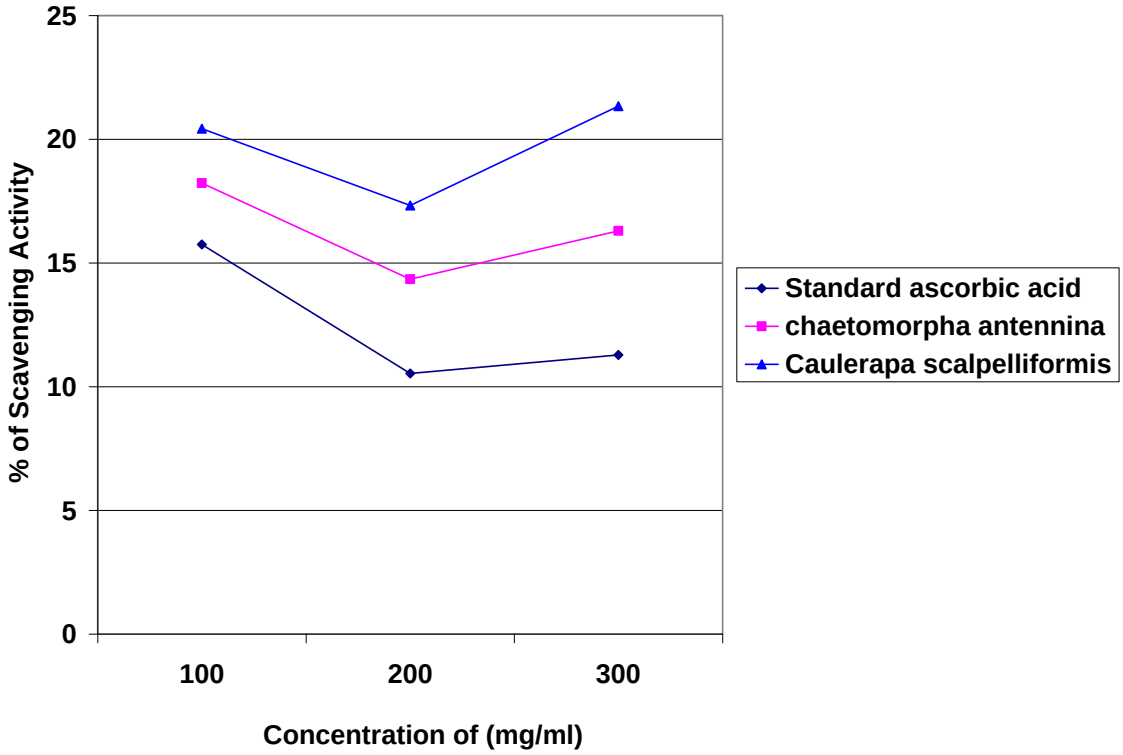


Figure 1: Inhibition % of DPPH radical scavenging activity of standard ascorbic acid and *Chaetomorpha antennina, Caulerapa scalpelliformis*

Therefore, it is suggested that further works may be performed on the isolation and identification of the antioxidant and antimicrobial components in Caulerpa scalpelliformis. for its industrial and pharmaceutical application.

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