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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *CHAETOMORPHA ANTENNINA* FROM RASTHACAUD COAST, TAMIL NADU, INDIA.

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ABSTRACT

Seaweeds of new and novel structures with useful biological activities are the main sources of drugs for medical treatments. Hence the present study was aimed to investigate the preliminary phytochemical analysis and antibacterial activity of *Chaetomorpha antennina* from Rasthacaud coast, Tamil Nadu, India. To make the study more authentic we prepared the crude extracts from the selected algae using different solvents namely, aqueous, petroleum ether, chloroform, ethanol and acetone. *C. antennina* extracts were tested against four Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and three Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis*). The phytochemical analysis showed the presence of flavonoids, glycosides, carbohydrates, tannins, quinones, coumarins, alkaloids, steroids, proteins, terpenoids, phytosterols and saponins. Carboxylic acid did not show any positive result. It was observed that acetone extract of the algae showed higher inhibitory activity than other solvent extracts. *E. coli*, *S. aureus* and *E. faecalis* were more susceptible pathogens among the tested organisms. From the overall results we conclude that this seaweed can be used against several diseases.

KEYWORDS: *Chaetomorpha antennina*, solvent extracts, phytochemical screening, antibacterial activity.

INTRODUCTION

Macroalgae or seaweed the most accessible marine resources of the coastal zone occupy potentially important place as a source of biomedical compounds. Seaweeds are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes, cyclic polysulphides, [1, 2] acrylic acid, saturated and unsaturated fatty acids.[3] Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from seaweeds and used in medicine and pharmacy.[4] Quality of protein and lipid in seaweeds are most acceptable for consumption compared to other vegetable mainly due to their high content in essential amino acid and relatively high level of unsaturated fatty acid.[5] Fresh and dried seaweeds are utilized as human food.[6] In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods. Researchers found that algae contain remarkable amount of components valuable for human health.[7-11] Synthetic drugs are not only expensive but are also often with adulterations and side effects. Therefore, there is a need to search for new strategies to control microbial infections.[12] Now-a-days, the use of antibiotics has increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs due to indiscriminate use of antibiotics. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alteration.[13, 14] Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential.[15] Antibiotic treatment of microbial diseases has been applied for many years. The prevention and treatment of these infectious diseases by applying products from macroalgae appears as a possible alternative. Hence, the interest in macroalgae has been increased during the last years.[16-18] Bacteriostatic and bactericidal activity of marine algae have been extensively studied by several researchers.[19-27] The first investigation on antibiotic activity of alga was carried out by Pratt *et al*.[28]

The antibacterial agents found in the algae include terpenoid, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketone and alkaline, cyclic polysulphides and fatty acids. In a number of marine algae antibacterial activities are attributed to the presence of acrylic acid.[29] Research showed that the antibacterial activity of algae is due to their ability to synthesize respectively nitrogen compounds and diterpenes in Chlorophyceae, mixed halogenated terpenes in Rhodophyceae and metabolites of aromatic origin in Phaeophyceae.[30] Among the macroalgae Chlorophyceae members form an important group

of seaweeds having rich source of potential new drugs.[31] So in this context, the present experimental study has been made to reveal the phytochemical constituents and antibacterial activity of *C. antennina* a Chlorophyceae member.

MATERIALS AND METHODS

Collection of seaweed

For screening of phytochemical constituents and antibacterial activity of *Chaetomorpha antennina* (Bory de Saint-Vincent) Kutzing, was collected from Rasthacaud coast (Lat. 08^0 08' 56.9178" N, Long. 77^0 36' 35.8704"E) of Kanyakumari district, Tamil Nadu, India. The collected samples were cleaned with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells. Then the samples were brought to the laboratory in sterile polythene bags. The samples were then washed thoroughly with tap and distilled water to remove the salt on the surface of the material. The samples were shade dried until constant weight obtained and ground in an electric mixer. The powdered samples were then stored in refrigerator for future use.

Preparation of extracts

The powdered sample (100g) was extracted in soxhlet apparatus using petroleum ether, chloroform, ethanol and acetone (1000 ml) as solvents for 8hrs at a temperature maintained not more than the boiling point of the solvent. Seaweeds were also extracted with hot-water for the preparation of aqueous solutions. The resultant crude extracts were filtered with Whatman No.1 filter paper. The filtrates obtained were concentrated under vacuum with a rotary evaporator at 40^oC to obtain the crude extracts. The crude extracts were collected in an air tight container and stored at 4^oC until use.

Phytochemical analysis

The preliminary phytochemical analysis was performed for the presence of flavonoids, glycosides, carbohydrates, tannins, quinones, coumarins, carboxylic acid, alkaloids, steroids, proteins, terpenoids, phytosterols and saponins using standard procedure.[32]

Test microorganisms

The bacterial strains were obtained from Vivek laboratories, Nagercoil, Tamil Nadu, India. Extracts were tested against three Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis*) and four Gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*).

Preparation of inoculum

The stock cultures were maintained at 4^{o}C on slant slopes of Nutrient Agar medium. Active cultures for the experiments were prepared by transferring a loopful of cells from the stock cultures to conical flasks containing Mueller Hinton Broth (MHB). These conical flasks were incubated at 30^{o}C for 24 hrs and were referred to as seeded broth.

Antibacterial activity

Agar disc diffusion metho

The antibacterial activity of aqueous, petroleum ether, chloroform, ethanol and acetone extracts of *C. antennina* was studied by agar disc diffusion method.[33] The strains that had been incubated for 24 hrs were used for this assay. The cultures of the organisms were seeded on Mueller Hinton agar plates by using sterilized cotton swabs. The agar surface was allowed to dry for five minutes. Then the discs (6 mm) impregnated with the seaweed extracts were placed on the swabbed plate using sterilized forceps. Chloramphenicol (30 mcg) disc was used as positive control. The plates were kept in an incubator at 37^{o}C for 24 hrs and the antibacterial activity was determined by measuring the zone of inhibition in millimeter by using graduated scale and recorded. Triplicates were maintained for each test.

Statistical analysis: The values of antibacterial activity of aqueous, petroleum ether, chloroform, ethanol and acetone extracts of *C. antennina* were expressed in mean ± standard deviation (n=3) for each sample.

RESULTS

Phytochemical screening: The present study carried out on the seaweed samples revealed the presence of medicinally active constituents. The phytochemical constituents of *C. antennina* was estimated and summarized in Table 1.

The phytochemical screening showed that *C. antennina* contained flavonoids, glycosides, carbohydrates, tannins, quinones, coumarins, alkaloids, steroids, proteins, terpenoids, phytosterols and saponins except carboxylic acid. Steroids and phytosterols were observed in all the five extracts followed by terpenoids in four extracts except chloroform. Next to those tannins, coumarins and proteins were present in three extracts. Among the five extracts acetone showed the presence of maximum number (eight) of compounds. Next to that aqueous and ethanol showed the presence of seven compounds.

Table 1: Preliminary phytochemical analysis of *Chaetomorpha antennina*

Phytochemicals	Aqueous	Petroleum ether	Chloroform	Ethanol	Acetone
Flavonoids	+	-	-	-	-
Glycosides	-	-	-	+	-
Carbohydrates	-	-	+	-	-
Tannins	-	-	+	+	+
Quinones	-	-	-	-	+
Coumarins	+	+	-	-	+
Carboxylic acid	-	-	-	-	-
Alkaloids	-	-	-	+	+
Steroids	+	+	+	+	+
Proteins	+	+	-	+	+
Terpenoids	+	+	-	+	+
Phytosterols	+	+	+	+	+
Saponins	+	-	-	-	-

+ = present; - = absent

Antibacterial activity: The investigation made on aqueous extract showed activity against *Pseudomonas aeruginosa* (14.33±0.47 mm). It has no activity against pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis*. The petroleum ether extract produced a maximum zone of 18.66±0.94 mm against *E. faecalis* and a minimum zone of 13.00±0.81 mm against *P. vulgaris*. The extract obtained using chloroform showed a maximum activity against *S. pyogenes* (22.00±0.81 mm) and minimum activity against *K. pneumoniae* and *S. aureus* (10.66±0.94 mm). No antibacterial activity against *P. aeruginosa* was observed.

Ethanolic extract didn't provoke zone of inhibition against *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *S. pyogenes* whereas, the maximum activity (22.66±0.94 mm) was recorded against *E. faecalis* and minimum activity (7.66±0.94 mm) was against *S. aureus*. Acetone extract pointed out maximum activity against pathogens *S. aureus* (29.33±0.47 mm) and *K. pnemoniae* (28.66±0.47 mm) and minimum activity against *E. faecalis* (11.66±0.47 mm) and *P. aeruginosa* (10.33±0.47 mm). Acetone and petroleum ether extracts showed excellent inhibition against bacterial pathogens which were well compared with standard drug chloramphenicol (30 mcg/disc). Chloroform extract showed remarkable antibacterial activity against all pathogens except *P. aeruginosa*. Ethanol extract inhibited *E. coli*, *S. aureus*, and *E. faecalis* whereas, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *S. pyogenes* were resistant to ethanolic extract. *E. coli*, *K. pneumoniae*, *P. vulgaris*, *S. aureus*, *S. pyogenes* and *E. faecalis* were not susceptible to aqueous extract and all the values were shown in Table 2.

Table 2: Antibacterial activity of *Chaetomorpha antennina* against bacterial pathogens

Solvents	Bacterial pathogens showing zone of inhibition (mm)						
	Escherichia coli	***Klebsiella pneumoniae***	***Proteus vulgaris***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Enterococcus faecalis***
Aqueous	-	-	-	14.33±0.47	-	-	-
Petroleum ether	15.33±0.47	16.33±0.47	13.00±0.81	14.00±0.81	16.00±0.81	15.66±0.94	18.66±0.94
Chloroform	20.00±0.81	10.66±0.94	21.33±0.94	-	10.66±0.94	22.00±0.81	20.00±0.81
Ethanol	9.66±0.47	-	-	-	7.66±0.94	-	22.66±0.94
Acetone	23.33±0.94	28.66±0.47	15.33±0.94	10.33±0.47	29.33±0.47	24.33±0.94	11.66±0.47
Positive control Chloramphenicol	36.33±0.47	26.66±0.94	21.33±0.94	-	22.66±0.94	24.33±0.47	28.33±0.47

- = No activity; mm = Millimeter, values are expressed as mean ± standard deviation (n=3)

DISCUSSSION

Earlier many studies were reported on the presence of different bioactive compounds and their antibacterial activity of marine algae.[34-36] In the present study flavonoids were observed in aqueous extract. Flavonoids were considered as active potentials in the field of medicine. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganism invitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall.[37] In the present study *C. antennina* showed the presence of glycosides in ethanol extract. Glycosides are known to lower the blood pressure.[38]

Tannins are known to possess general antimicrobial and antioxidant activities.[39] Chloroform, ethanol and acetone extracts of the present study revealed the presence of tannins which suggest that *C. antennina* can be used as antibacterial agents. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria.

[40] In the present study alkaloids were observed in ethanol and acetone extract of *C. antennina*. Saponins possess specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effect.[41] Saponins were reported only in aqueous extract in the present study whereas steroids were noticed in all the five extracts. Steroids have been reported to have antibacterial properties.[42] Active ingredient present in marine algae can cure diseases. The present study carried on *C. antennina* revealed the presence of medicinally active constituents. Phytochemicals such as flavonoids, glycosides, carbohydrates, tannins, quinones, coumarins, alkaloids, steroids, proteins, terpenoids, phytosterols and saponins present in the seaweed could be responsible for the observed antibacterial activity.

The seaweeds have an effective antibacterial activity against most of the human pathogenic bacteria. In this study, acetone extract of the tested algae was more active than petroleum ether, chloroform, ethanol and aqueous extracts against the bacterial pathogens. Several earlier workers have used different solvents to extract bioactive principles from seaweeds and arrived at varying conclusions. Rao and Karmarkar [43] confirmed diethyl ether as suitable solvent to extract active compounds. Martinez-Nadal *et al.*[44] mentioned that benzene and diethyl ether were the suitable solvents for extracting of antibiotic principles. Sastry and Rao [45] and Febles *et al.*[46] reported that chloroform extract exhibited the strongest activity. Tuney *et al.*[47] reported that diethyl ether caused better halo-zones than methanol, acetone and ethanol. Lavanya and Veerappan [48] confirmed methanol as suitable solvent in extracting majority of the algae.

The results of the inhibitory effect were greater in the acetone extract than the petroleum ether, chloroform, ethanol and aqueous extracts for all organisms in the present study. This indicates that the metabolites responsible for such effect are soluble in this solvent, supporting the results from Hornesy and Hide, [49] Sreenivasa Rao and Parekh, [34] Parekh *et al*., [50] Ballantine *et al*., [51] Vidyavathi and Sridhar, [35] De Lara-Isassi *et al*., [52] Wefky and Ghobrial, [53] Fareed and Khairy, [54] Osman *et al.*, [55] Henry Borbon *et al.*[56] and Kayalvizhi *et al.*[57] who indicate the bioassay conducted with marine algae using acetone as solvent extraction showed a better response as antibiotic. This may indicate that the components responsible of inhibitory activity are generally phenol groups, fatty acids and unsaponifiable lipids.[34]

Petroleum ether ranked the second order sustaining high inhibition zone diameters. Next to this, chloroform and ethanol extracts showed the inhibition zones. On the other hand aqueous extract showed antibacterial activity only against *P. aeruginosa*. This could be probably due to the difference in the solubility of bioactive metabolites in the corresponding solvents. It is clear that the use of organic solvents always provides a higher efficiency in extracting antibacterial activities as compared to water extract.[58-60]

Kandhasamy and Arunachalam [61] reported that green algae were more active when compared to other groups which confirm to the high antibacterial activity from the acetone extract of *C. antennina* in this study. The present results are in accordance with those of Boonchum *et al*.[62] who reported that *Halimeda macroloba* (green algae) showed high antibacterial activity and also agreed with that of Fareed and Khairy [54] who showed that *Ulva lactuca* (Chlorophyceae) were more active than *Jania rubens* (Rhodophyceae). Osman *et al*.[55] reported that *Ulva fasciata* (green algae) was more active than other groups of algae. In our study the chloroform extract showed inhibition against *E. coli*, *K. pneumoniae*, *P. vulgaris*, *S. aureus*, *S. pyogenes* and *E. faecalis*. But *P. aeruginosa* was resistant to this extract. The findings of this study coincide with the findings of Prasanna Latha and Hema Latha [63] that chloroform extract of *C. antennina* showed inhibition against *S. aureus*, *E. coli*, and *P. vulgaris*. Siva Kumar and Safhi [64] analysed petroleum ether extract of C. antennina found that the algae showed high activity against *E. coli* and *P. aeruginosa*. Similarly our work also supports the earlier findings. Manikandan *et al*.[65] reported that methanol extract of *C. antennina* was unable to inhibit *E. coli*, *K. pneumoniae* and *P. aeruginosa*. In contrast, our results revealed that acetone and petroleum ether extracts inhibit *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Sumathi and Krishnaveni [66] reported that methanol extract of *C. antennina* was able to inhibit *K. pneumoniae* (10 mm) and *E. coli* (11 mm).

Petroleum ether extract of present study showed inhibition against *K. pneumoniae*. This result differs from Siva Kumar and Safhi [64] who recorded that petroleum ether extract was unable to inhibit *K. pneumoniae*. Premalatha *et al*.[67] reported that ethanol extract of *C. antennina* showed inhibition against *K. pneumoniae* (8 mm), *Proteus* sp. (6 mm), *P. aeruginosa* (20 mm) whereas, our study showed that ethanol extract was unable to inhibit these three pathogens. The differences between the results of the present investigation and results of other studies may be due to the production of bioactive compounds related to the seasons, method and organic solvents used in assay methods.[23, 60, 68, 69] Natural products play an

important role in drug development programs of the pharmaceutical industry.[70, 71] Present study confirms the ability of *C. antennina* in such application.

CONCLUSION

The seaweed *C. antennina* was analyzed for its phytochemical constituents and antibacterial activity. The phytochemical analysis showed the presence of flavonoids, glycosides, carbohydrates, tannins, quinones, coumarins, alkaloids, steroids, proteins, terpenoids, phytosterols and saponins. Carboxylic acid was absent in the algae. In antibacterial activity highest zone of inhibition (29.33±0.47 mm) was observed in acetone extract of *C. antennina* against *S. aureus*. *E. coli*, *S. aureus* and *E. faeclis* were the most susceptible bacterial pathogens followed by *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *S. pyogenes*. The tested algae showed more or less equal zone of inhibition or slightly greater against tested pathogens when compared with positive control Chloramphenicol impregnated discs. Acetone extract of *C. antennina* showed good antibacterial activity against seven bacterial pathogens used. The present study suggests that *C. antennina* contains phytochemical constituents and possess antibacterial activity against bacterial pathogens. This could lead to the development of new drug for the treatment of bacterial infections. Thus require further studies to identify the compounds responsible for these activities.

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