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Biomedical Potential of Seaweeds from Gulf of Mannar Coastal Waters

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Abstract: The present study has been focused on to investigate the role of extracts of seaweeds *Enteromorpha intestinalis* and *Sarconema furcellatum* against human pathogens. The crude extract of the seaweeds have shown promising activity against most of the screened pathogens. The green algae *Enteromorpha intestinalis* was very active against human pathogens and could be a good source for the isolation of novel compounds. In partitioning crude extract portioned between ethylacetate, butanol and water. The activity of each phase was screened using disc diffusion method. Among them ethyl acetate phase was found to have good activity indicated the nonpolar nature of the active compound.

Key words: Marine Algae · Human Pathogens · Southeastern India

INTRODUCTION

Marine organism especially the sedentary forms possesses characteristic chemical defense mechanisms to ward off the growth of fouling organism on their surfaces. Benthic organisms have developed a great variety of potential defenses against fouling organisms, including possession of spines, surface sloughing [1], production of mucus and the production of secondary metabolite [2]. Many tropical macroalgae combines chemical and morphological defenses to persist in reef habitats where herbivores are numerous and diverse [3]. Chemical defense exhibited by organism can be divided into 2 groups. They are constitutive chemical defense that is always prominent in the organism and inducible chemical defense, which is induced after attack by the predator [4,5].

Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites with good bioactivity, cytotoxic [6], nematicidal and fungicidal [7] and hypoglycemic activities [8]. Liquid concentration of brown algae *Ecklonia maxima* significantly reduced the root knot infestation and increased growth of tomato plant.Marine algae have been screened extensively for the isolation life saving drugs or biologically active substances. The greatest variety of natural products is probably found among Rhodophyta in which all known classes of compounds are represented many of which are halogenated [9]. The genus Laurenicia produce amazing array of complex terpenoids and acetogens, possibly making it the world's most chemically complex genus. Brown algae produce larger quantity of phenolic compounds reported by Ragan and Glombitza [10] that effectively deters feeding by marine herbivores. *Chaetomorpha linoides* have been extensively studied are known to produce, many of which are broad spectrum feeding deterrents against herbivores or show other biological activities. Special attention has been paid for antiviral and or antifungal activities related to marine algae against several pathogens [11].

The objective of this study is to evaluate the antibacterial activity of seaweeds against human pathogens. Seaweeds are reported to possess secondary metabolites having various properties like antifouling, antideterrent and antimicrobial activity. Though many works have been carried out in temperate and tropical waters, the studies pertaining to the said subject are very limited in India. So, it has been planned in the present study to investigate the antibacterial activity exhibited by

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the marine macroalgae *Enteromorpha intestinalis* and *Sarconema furcellatum* against human pathogenic bacteria. The other objective of the study is to localize and to assess the polarity of the active compounds through partitioning to obtain active fraction and to explore the possibility of finding out new drug leads to combat the ever increasing drug resistance and emerging new diseases.

MATERIALS AND METHODS

Study Area: The seaweeds were collected from Tuticorin coast (Lat. 8°45 N; Long. 78°10 E) of Gulf of Mannar, Southeast Coast of India (Fig. 1). Gulf of Mannar, a marine biosphere reserve established in 1989, harbours biodiversity of global significance and is unique for coral reef, seaweed and sea grass ecosystems. The seaweeds, which inhabited exclusively at the intertidal rocky substratum, were selected for the collection.

Extract Preparation: Algal samples were cleaned of epiphytes extraneous matter and necrotic parts were removed. Plants were washed with sea water and then in fresh water. Then they were transported to the laboratory in sterile polythene bags. In the lab they were rinsed with sterile sea water and were shade dried at room temperature for 24 hrs. They are cut into pieces and powdered is a mixer grinder. The powdered samples were extracted with solvents (Acetone, Diethylether and Butanol) separately which was cold steeped overnight at-18°C. It was filtered through Whatman No.1 filter paper and then evaporated and concentrated [12]. The crude extracts were tested for their antimicrobial activity against the ten human pathogens.

Test microorganisms were clinical isolates and maintained in the laboratory. The pathogen includes *Staphylococcus* sp*, Streptococcus* sp*, Bacillus subtillis, Escherichia coli, Pseudomonas* sp*, Shigella* sp*, Proteus* sp*, Salmonella* sp*, Bacillus cereus* and *Bordetella* sp. The pathogenic bacteria were cultured individually in nutrient broth at room temperature for 18 hrs before used for assay.

Antibacterial Assay: Antibacterial activity was evaluated by agar disc diffusion method [13]. 50mg of crude extract was dissolved in 1ml of respective solvent. From this stock solution 10μl of each extract was loaded on sterile discs (6mm diameter) and air dried. After drying, discs were placed on the Muller-Hinton agar inoculated with the culture and they were kept for incubation at room temperature.

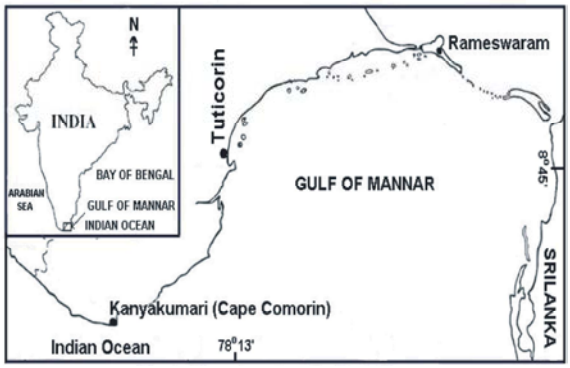

Fig. 1:

Partial Purification: Partitioning of the crude extract in the preliminary step for the isolation of active compounds and it is considered as the first chromatographic step. The sequential gradient partitioning with solvents will have the fractions with compounds distributed according to their polarity. Normally low or medium polar compound usually contains lipophilic organic compounds and high polar fractions contain water soluble organic compounds.

The extract with high activity is partitioned between ethyl acetate and water. This water phase is subsequently partitioned again with n-butanol to localize and to assess the polarity of active substance [14]. Then the three phases were collected separately, evaporated, concentrated and screened against ten human pathogenic bacteria.

RESULTS

In the present study, extracts of two seaweeds *Enteromorpha intestinalis* and *Sarconema furcellatum* with solvents acetone, diethyl ether and butanol were found to show antimicrobial activity against human pathogens. They were tested against the bacterial pathogens by agar diffusion method. The results of preliminary screening tests were summarized in Table 1, which revealed that they possess anti-bacterial activity.

Invariably all the crude extract of the seaweeds exhibited antibacterial activity against the pathogens tested (Table 1). All the crude extracts inhibited the growth of the pathogens except one or two pathogens. Crude extracts of both the seaweeds showed high inhibitory activity against *Bacillus subtilis*. Bacterial pathogen *Pseudomonas* sp was resistant to all extracts. Particularly the acetone extract of *Enteromorpha intestinalis* showed high activity against *Staphylococcus* (5 mm) and *Bacillus subtilis* (6 mm). Over all the activity was recorded better with acetone and diethyl ether,

Table 1: Antibacterial activity of crude extract of seaweeds

		Enteromorpha intestinalis			*Sarconema furcellatum*		
		(Zone of inhibition-mm)					
S. No	Pathogenic organism	Acetone	Diethyl ether	Butanol	Acetone	Diethyl ether	Butanol
1	*Staphylococcus* sp	5	2	Trace	3	3	-
2	*Streptococcus* sp	2	2	-	3	2	2
3	*Escherichia coli*	3	Trace	-	4	3	3
4	*Pseudomonas* sp	-	-	-	-	-	-
5	*Bacillus subtilis*	6	2	Trace	2	Trace	-
6	*Shigella* sp	4	2	-	3	Trace	-
7	*Proteus* sp	2	2	-	2	2	-
8	*Salmonella* sp	4	Trace	2	2	2	Trace
9	*Bacillus cereus*	3	3	Trace	3	2	-
10	*Bordetella* sp	2	Trace	-	2	-	-

Table 2: Antibacterial activity of partitioned extract

		(Zone of inhibition-mm)	
S. No	Pathogenic organism	Ethyl Acetate	Butanol
1	*Staphylococcus* sp	4	-
2	*Streptococcus* sp	4	-
3	*Escherichia coli*	3	-
4	*Pseudomonas* sp	-	-
5	*Bacillus subtilis*	5	4
6	*Shigella* sp	4	-
7	*Proteus* sp	3	-
8	*Salmonella* sp	2	-
9	*Bacillus cereus*	2	-
10	*Bordetella* sp	-	-

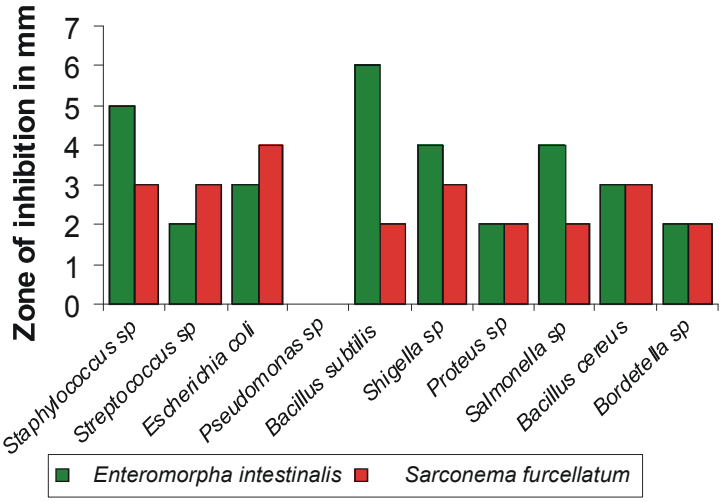

Fig. 2: Antibacterial activity of acetone extract of seaweeds against Human pathogens

the activity was very low in the case of butanol. Next to *Staphylococcus* sp, *Streptococcus* sp. was susceptible to all the extract used in the present study. The partitioned extract showed good activity with that of the ethylacetate phase except Pseudomonas sp. and *Bordetella* sp. The activity of all the other pathogen was summarized in Table 2. The activity of the acetone extract of both the seaweeds has been compared in figure 2.

DISCUSSION

The exhibition of activity against pathogenic bacteria by the extracts of the seaweeds *Enteromorpha intestinalis* and *Sarconema furcellatum* indicated the presence of antimicrobial activity having an ecological role of seaweeds which coincides with the observation of Paul and finical, [15], where the secondary metabolites from seaweeds shown broad spectrum bioactiity. Marine algae are known to produce antimicrobial substances [15] and their production varied with season. Ragan and Glombitza [8] opined that the secondary metabolite production in seaweeds have shown diurnal and seasonal variation. The variation in antimicrobial activity may also be due to the method of extraction, solvent, used in extraction and season at which samples were collected. Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kind of solvent is the most effective and suitable for extraction of seaweeds. A few workers tried using different solvents and made comparison. Rosett and Srivastava [16] found similar antibacterial activity with acetone, ethyl-ether, methanol and acetic acid. The results from the present screening revealed that the strongest antibacterial activities were exhibited by the crude acetone extract and the least by the butanol extract. In some species (such as *Gelidium amansii*) the inhibitory activity was observed only in the extracts obtained with one kind of solvent but not in extract obtained with other solvents, which may suggest that a particular solvent is required to extract some antimicrobial substances within the algal plants. The lack of activity in other extract in the disc diffusion assay doesn't mean that the extracts have no active ingredients. Though the screening by disc diffusion assay has its own advent as it is simple, less time consuming and requires only a small quantity of material.

The results in the present study revealed that Gram-positive organisms were more susceptible to the crude extract of algae used coinciding with the report of Tuney *et al*. [17] and is similar to the observation of Taskin *et al.* [18] indicating the difference in their cell wall composition[19]. In Gram-negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics [20]. The presence of thick murine layer in the cell wall also prevents the entry of the inhibitors [21].

Reichelt and Borowitzka [22] screened many species of algae for their antibacterial activity. They reported that the members of red algae exhibited higher activity. In contrast, green algae (Chlorophyceae) were the most active. In this study *Enteromorpha intestinalis* member of green algae was most active.

The present result coincides with Rao and Parekh [23] where the extracts of *Enteromorpha intestinalis* were active throughout the year with the peak during the winter season. The sequential gradient partitioning with solvents will have the fractions with compounds distributed according to their polarity. In general lipophilic non-polar compound such as terpenoids, acetogenins and compounds of mixed biosynthesis occurs in relatively low concentration (0.2-2.0% of algal dry weight) but polar polyphenolics can occur in concentration as high as 15% of algal dry mass [8]. The partitioning of the acetone extract shown wide spectrum activity against pathogenic bacteria in the ethyl acetate phase indicating the non-polar nature of the active substance, though all the three extracts have shown activity and only the acetone extract which showed higher activity was taken for partitioning. Hence the presence of other polar compounds in the seaweeds is not ruled out.

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