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Antibacterial Properties of *Enteromorpha flexuosa* (Wulfen) from the Gulf of Mannar-Southeast Coast of India

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ABSTRACT

The antibacterial properties of chloroform, methanol, ethanol and water extracts of marine algae *Enteromorpha flexuosa* (Wulfen, Chlorophyta) from Mandapam region of Gulf of Mannar Southeast coast of India were tested against Gram-positive strains such as *Staphylococcus aureus, Bacillus cereus* and *Streptococcus pyogenes* Gram-negative strains *Escharichia coli*, *Salmonella typhimurium, Proteus mirabilis* and *Klebsiella pneumoniae*. The preliminary phytochemical analysis of extract from *Enteromorpha flexuosa* showed the presence of biologically active compounds namely alkaloids, flavonoids, steroids, terpenoids cardiac glycosides and tannins. Results demonstrated that methanolic extract of *Enteromorpha flexuosa* exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. It was observed that the extract of *Enteromorpha flexuosa* recorded maximum activity against *Streptococcus pyogenes*.

Keywords- *Enteromorpha flexuosa*, Antibacterial activity, Crude extracts, Agar well diffusion.

INTRODUCTION

Enteromorpha flexuosa (Wulfen, Class: Chlorphyceae, Order: Ulvales, Family: Ulvaceae) is an abundantly growing green seaweed in coastal seashore of South India. Marine macro algae are rich and varied source of bioactive natural products and have been studied as potential biocidal and pharmaceutical agents[1]. In recent years, there are several reports of macro algae derived compounds that have a wide range of biological activities such as antibacterial, anti-fungal, antiviral, anti-neoplastic, antifouling, anti-inflammatory, anti-turmeric, cytotoxic and anti-diabetic[2]. At present seaweeds constitute commercially important marine renewable resources which are providing valuable thoughts for the development of new drugs against cancer, microbial infections and inflammations[3]. Marine algae have been screened broadly to isolate life saving drugs or biologically active substances all over the world[4].

Bacterial infections cause high rate of mortality in human population and other

organisms. *E. coli* and *Staphylococcus aureus* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella sps* cause diarrhea and typhoid fever[5,6]. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. Now days, the use of antibiotics increased significantly due to heavy infections and then pathogenic bacteria becoming resistance to drugs[7].

Bacteria are common disease agents in humans, as shown by the wide clinical use of antibiotics. Antibiotics have been called 'miracle drugs' for a reason. Before antibiotics were discovered it was the 'dark age' of medicine. For thousands of years doctors used many types of plants, fungi and lichen to try heal infections without knowing how it worked. As soon as antibiotics were discovered medicine, industry and farming were revolutionized. Antibiotics have really changed the way that medicine is practiced. Antibiotics are one of the most frequently prescribed medications in modern times. In this present study, seaweed was selected and used for *in vitro* screening of antimicrobial activity.

Marine algae use targeted antimicrobial chemical defense strategies by eliciting secondary metabolites, which are important in ecological interactions between marine macro organisms and microorganisms[8]. The extracts of many species of marine algae inhibit the growth of Gram-positive and Gram negative bacteria[9]. Various levels of antimicrobial activity were due to different solubility behavior of secondary metabolites which were influenced by season and geographical distribution of the species[10]. In the several species, different substances have been found in the same algae. Study on antimicrobial activity of seaweed has been reported wide in India[11].

Even though marine algae from many coastal regions of India were investigated for antimicrobial activity, majority of marine algae from Gulf of Mannar, South east coast of Tamilnadu, India were left unexplored for bioactive substances. In the present study, we describe the antibacterial characteristics of chloroform, ethanol, methanol and water extracts of marine algae *Enteromorpha flexuosa* obtained from the coast of Gulf of Mannar.

MATERIALS AND METHODS

Sample Collection

In the present study, *Enteromorpha flexuosa* (Wulfen) macro algae was collected from Mandapam coastal region (78°8′E, 9°17′N), in Gulf of Mannar, Tamilnadu, South India on low tide during December 2012 and immediately brought to the laboratory in polythene bags and washed several times with seawater to remove sand, mud and attached fauna. The algae were cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, algae were dried in shade at room temperature for one week. The dried algae materials were homogenized to fine powder and further subjected to extraction.

Crude Extraction

500g of powdered algae sample were taken and extracted successively with different solvents in the order of their polarity chloroform, ethanol, methanol and water using soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to get their corresponding residues. The algae extracts were further subjected for antimicrobial activity by agar well diffusion method.

Microorganisms Tested

The following strains of bacteria were tested; *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Streptococcus pyogenes* (MTCC 1927), *Escherichia coli* (MTCC 443), *Klebsiella*

pneumonia (MTCC 432), *Proteus mirabilis* (MTCC 1429) and *Salmonella typhimurium* (MTCC 98) were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures were maintained in nutrient agar (High Media, India) slants at 4°C and were sub-cultured before use. The bacteria studied are clinically important ones causing several infections and it is essential to overcome them through some active therapeutic agents.

Antibacterial Assay

In the present study, the antibacterial activity of the *Enteromorpha flexuosa* was studied by the agar well diffusion method[12]. The chloroform, ethanol, methanol, and water extracts of the collected test samples were tested in two dose levels of 300 and 500mg/ml. The nutrient agar medium prepared was inoculated with 18 hours old cultures of the above mentioned test organisms and were transferred into sterile 15cm diameter Petri dishes. The medium in the plates were allowed to set at room temperature for about 10 minutes and allowed to solidify in a refrigerator for about 30 minutes. 5 cups of 6mm diameter were made in each plate in equal distance. Stock solutions of the test residual extract were prepared in concentrations of 300mg/ml and 500mg/ml. 50µl of each concentration were loaded in each well with sterile pipettes. In each plate one well was used for control and standard. Antibiotic Chloramphenicol (100µg/ml) was used as standard and respective solvents were used as control. The Petri dishes thus prepared were incubated for 16hrs at 37°C and were later examined by measuring the zones of inhibition with the zonal scale and the results were tabulated.

RESULTS

Four different extracts of *Enteromorpha flexuosa* were tested for their anti-bacterial activity against strains of Gram positive, Gram negative bacteria using agar well diffusion method. The result of antimicrobial activity against tested pathogens was tabulated (Table. 2). The crude extracts of *Enteromorpha flexuosa* in different solvents exhibited diverse antibacterial activities. The methanol extract of *Enteromorpha flexuosa* was most active against all tested pathogens followed by chloroform and ethanol extracts, the minimum with water extracts. *Streptococcus pyogenes* growth was highly inhibited (21mm at 300mg/ml and 28mm at 500mg/ml) by the methanol extracts of *Enteromorpha flexuosa* when compared with other extracts. The antimicrobial activity of *Enteromorpha flexuosa* was observed to be dose dependent manner used for all four extracts. Highest antibacterial activity was exhibited in 500mg/ml than 300mg/ml dose level against all the tested pathogens.

DISCUSSION

Marine algae synthesize active constituents which are used in conventional and complementary medicine. Different varieties of marine algae were reported to have active ingredients that can cure diseases. Currently large population prefers to use remedies of natural origin for curing illness as these claimed to produce less side effects[13]. The present study was focused on *Enteromorpha flexuosa* for the presence of phytochemical constitutes and antibacterial activity against Gram-positive and Gram-negative bacteria.

The evolution of antibiotic-resistant pathogenic bacterium has stimulated the search for alternative antibacterial agents from alternative sources including sources from the ocean. The power of marine macro algae have been realized for thousands of years and its potential as producers of

pharmaceutical products have been reviewed[3].

The results of the present study clearly showed that marine algae extracts showed antibacterial activity against tested pathogenic bacterial strains including antibiotic resistant strains. The effectiveness of the active compounds present in the methanol extracts of *Enteromorpha flexuosa* causes the production of growth inhibition zones that appear as clear areas surrounding the wells. Antibacterial activity may be due to active components which are present in the extracts. Among tested pathogens, the methanol extracts of *Enteromorpha flexuosa* showed maximum growth inhibiting activity against Gram-positive bacteria especially *Staphylococcus aureus,* while chloroform extract and ethanol extract of *Enteromorpha flexuosa* showed moderate activity of all Gram-positive and Gram-negative bacteria. Water extracts of *Enteromorpha flexuosa* was showed mild activity against Gram-positive and Gram-negative pathogens. A dose depend increase in the antibacterial activity was observed in all the extracts of *Enteromorpha flexuosa* against the tested pathogens. The present study revealed that Gram-positive organisms were more susceptible to the crude extracts of *Enteromorpha flexuosa* used.

Gram-positive bacteria were more effectively controlled by the extracts of the algae used than Gram-negative bacteria[14]. Similar observations indicating that the more susceptibility of Gram-positive bacteria to the algal extracts is due to the differences in their cell wall structure and the composition of the cell wall[15]. The principle strength of the active components depends on the use of a suitable solvent to extract it[16].

Hornesy and Hide[17] reported that crude extracts of marine algal species showed inhibitory activity against pathogenic bacteria. But variation in antibacterial activity is due to the method of extraction, solvent used in extraction and season at which samples were collected. Previous reports on red algae exhibited high antibacterial activity[18,19], in contrast, green algae (Chlorophyceae), *Enteromorpha flexuosa* were also effective.

Results from this study revealed that the crude methanol extract of *Enteromorpha flexuosa* contain certain constituents with antibacterial property which enables the extract to overcome the barrier in Gram-negative cell wall[20]. In addition, these results form an absolute basis for selection of the plant for additional phytochemical and pharmacological investigations need biologically active molecules for novel antimicrobial agents.

CONCLUSION

Marine algae provide a rich source of chemically diverse compounds that can be used to develop various therapeutic agents. Certain marine algae reported for their antibacterial activities. In this work, we were able to show that *Enteromorpha flexuosa* can be used for development of anti-microbial agents in pharmacology and medicine industries. Our results also open that the antibacterial activities of the marine macroalgae may be accredited to the occurrence of diverse antibacterial compounds. Still a detail study in this field is required for production of antibacterial compounds from marine macroalgae.

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Table 1. Microbial strains used and their infection in human

Bacteria	Infections
Staphylococcus aureus (MTCC 96)	Superficial infection, Deep infection, Septicemia, Brain abscess, Food poisoning, Skin exfoliation, Toxic shock syndrome.
Bacillus subtilis (MTCC 121)	Food poisoning, CNS shunts infection, Pneumonitis, Entocartidis and Bacteraemia.
Streptococcus pyogenes (MTCC 1927)	Erysipelas, Glomerulonephritis, Impetigo, Necrotizing fasciitis, Pharyngitis, Pneumonia (E), Quinsy, Scarlet fever, Sinusitis, Strep throat, Tonsillitis and Toxic shock syndrome
Escherichia coli (MTCC 443)	Urinary tract infection, Gastro intestinal diseases, Traveler's diarrhea, Infant diarrhea, Hemorrhagic colitis, Septicemia and pyogenic infection
Klebsiella pneumonia (MTCC 432)	Pneumonia, Urinary infection, Septicemia, Wound infection and Epidemic diarrhea
Proteus mirabilis (MTCC 1429)	Urinary tract infection, Wound infection, Septicemia and Acute otitis media
Salmonella typhimurium (MTCC 98)	Food poisoning and Enteric fever

Table 2. Antibacterial activity of crude extract of *Enteromorpha flexuosa* (Wulfen)

Test Sample	Concentration (mg/ml)	Pathogens used and Zone of growth inhibition size (mm)						
		Gram positive organisms			Gram negative organism			
		Staphylococcus aureus	*Bacillus cereus*	*Streptococcus pyogenes*	*Salmonella typhimurium*	*Escherichia coli*	*Proteus mirabilis*	*Klebsiella pneumonia*
Chloroform extract	300 500	18 22	12 17	20 25	6 9	16 21	2 4	2 2
Ethanol extract	300 500	16 18	11 14	18 22	4 7	12 14	- 2	- -
Methanol extract	300 500	18 24	14 19	24 28	3 8	14 19	- 2	4 6
Water extract	300 500	6 10	4 8	6 9	- -	4 6	- -	- -
Standard/ Control	25	23	20	22	25	20	21	22