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Tropical marine macroalgae as potential sources for antibacterial activity against clinical pathogen

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ABSTRACT

To screen the antibacterial efficacy of various solvent extracts of marine algae such as *Ulva lactua* and *Hypnea musciformis* against some selected gram-positive and gram-negative human pathogenic bacteria. Crude extracts were prepared from the selected marine algae using different solvents namely, chloroform, n-butyl alcohol, and methanol and were tested for their antibacterial activity against human pathogenic bacteria using agar cup plate diffusion method. Minimum inhibitory concentration (MIC) was also performed for selected solvent extracts for all the bacterial species. A suitable positive control was also maintained. Among the two marine algae screened *Ulva lactua* and *Hypnea musciformis* were found to be more active N-butyl alcoholic extracts will be showed highest antibacterial activity against pathogens when compared with standard Amoxicilin (Positive control). It was observed that the N-butyl alcoholic extracts of all the two marine algae showed higher inhibitory activity for the selected bacterial species than other solvent extracts. The results revealed that the crude N-butyl alcoholic extracts seem to be a good source material in identifying the effective pure antibacterial compound(s) in all the three marine algae and particularly, *Hypnea musciformis*. The present study showed that the N-butyl alcoholic extracts of marine algae such as *Ulva lactua* and *Hypnea musciformis* exhibited good antimicrobial activity. But the N-butyl alcoholic extracts of *Hypnea musciformis* possessed highest antibacterial activity than others and so it could be useful in seeking active principles against clinical pathogenic bacteria.

Introduction

The macroalgae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals [1]. Since presence of antibacterial compounds in marine algae has been known since late 1800's [2] and the use of algal extracts as antiseptic has been documented since 1937 [3]. A number of screening studies dealing with the antibacterial activity of marine algae showed variations in the production of antimicrobial compounds [2]. Brazilian red algae have been found to have phenolic substances. Oxidative stress is an important factor in the pathological genesis, from cancer to cardiovascular and degenerative disease [4, 5]. Among the marine organisms, seaweed represents one of the richest sources of natural antioxidants and antimicrobials. [6,7] Cox *et al.*, 2010). They are an excellent source of vitamins such as A, B_l, B_{12}, C, D and E, riboflavin, niacin, pantothanic acid and folic acid as well as minerals such as Ca, P, Na, K [8]. The fat content of seaweeds accounts for 1-6 g/100 g dry weight with some brown varieties, such as *Hizikia* sp. and *Arame*, having a fat content as low as 0.7-0.9 g/ 100 g dry weight [9].

In particular, marine macroalgae are subjected to the phenomenon of epibiosis (biofouling) and the synthesis of secondary metabolites with different activities toward bacteria, fungi and protozoa is useful to maintain their surface free of this kind of epibionts [10]. Hence, marine macroalgae represent a promising valuable source of new compounds for drug development [11]. In recent years pathogenic bacteria resistant to multiple drugs have become a worldwide emergence so, the discovery of new antibacterial compounds, as suitable substitutes to conventional antibiotics, might be a possible solution to this problem. Seaweeds could represent a potential source of new antimicrobial compounds. One of the first observation regarding seaweeds antibiotic activities was reported by Pratt et al. [12]. Successively, some antibacterial compounds have been isolated and characterized essentially as fatty acids, peptides, nucleosides and pigment derivatives [13].

There are a number of reports regarding the medicinal importance of sea weeds belonging to Phaeophyceae, Rhodophyceae and Chlorophyceae from all over the world [14-17]. Many studies were reported earlier on the anti microbial study of marine algae [18-20]. Therefore, the aim of the present study was to investigate the antimicrobial activity of extracts of marine algae against five pathogenic bacteria that are often the cause of bacterial diseases. The investigated seaweeds were selected in according to their potential as component of integrated systems, as sources of pharmaceuticals, fine chemicals or cosmetics and as fish feed. The possible use of active seaweeds for prevention or treatment of the bacterial diseases.

Materials and Methods:

Sample collection

The seaweeds (*Ulva lactua* and *Hypnea musciformis)* were collected in bulk quantity from coastal areas of Mimisal, Pudukottai district, Tamil Nadu, India. Seaweed species exposed on sand and rocks were collected in plastic bags and brought to

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the laboratory. Each species was washed thoroughly with running water to remove epiphytes; animal castings, attached debris and sand particles and the final washings were done using distilled water and dried under shade. Shade dried samples were grounded to fine powder. The powdered samples were then stored in refrigerator for further use.

Extraction procedure

The 50gms of each powdered sample were taken and extracted successively with different solvents in the order of their polarity (chloroform, n-butyl alcohol, and methanol) using Soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to get their corresponding residues.

Bacterial strains

Antibacterial activity, the test organisms *Enterobacter aerogens*, *Pseudomnas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aereus* and *Bacillus cereus* were collected from the Department of Microbiology, Sri Mad Andavan College of Arts and Science, Thiruvanaikovil, Thiruchirappalli, Tamil Nadu, India.

Antibacterial activity assay

In the present study, the antibacterial activities of the seaweed were studied by the agar cup plate diffusion method. The chloroform, n-butyl alcoholic and methanolic extracts of the collected test samples were tested in three dose levels of 100 µg/ml, 150 µg/ml, 200 µg/ml,and 250 µg/ml,respectively. The nutient agar medium prepared was inoculated with 18 hours old cultures of the above mentioned test organisms and were transferred in to sterile 15 cm diameter petridishes. 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. 6 cups of 6mm diameter were add in each plate at equal distance. 0.5mg of crude extact was dissolved in 1ml of DMSO. From the stock solutions of the test residual extract were prepared in concentrations of 100 µg/ml, 150 µg/ml, 200 µg/ml,and 250 µg/ml. 100 µg/ml of each concentration were placed in the cups with sterile pipets. In each plate one cup was used for control and standard. Antibiotic amoxciline 250 µg/ml was used as standard and DMSO as control.The petridishes thus prepared were incubated for 16 hours at 37.2◦C and were later examined by measuring the zones of inhibition with zonal scale and the results were tabulated.

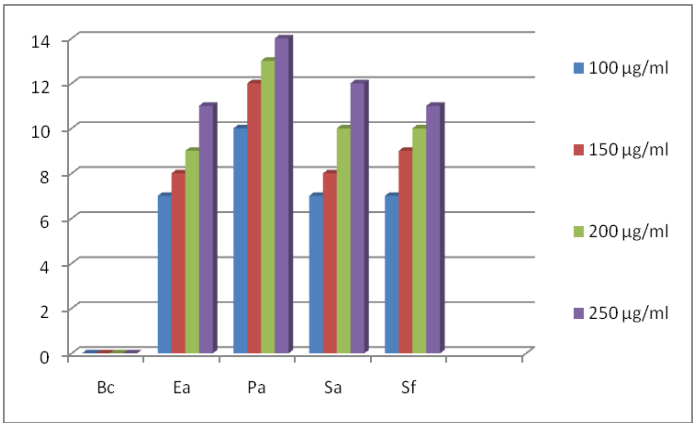
Results

Antimicrobial assay

Ulva lactuca

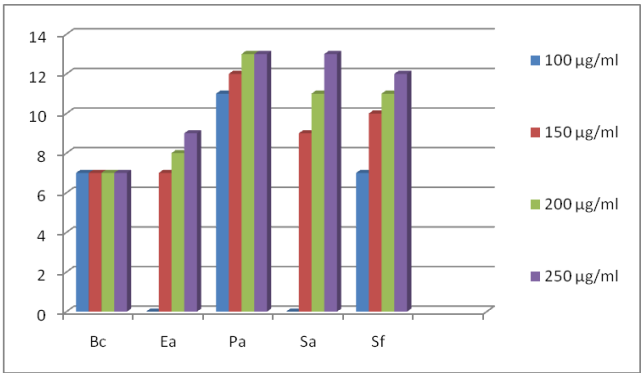
In the present investigation of marine algal extracts (*Ulva lactuca*, *Hypnea muciformis)* were tested against the bacterial pathogens by agar cup plate method. The results of the tests were summarised (Table 1and 2). Which revealed that except few extracts all other extracts possess antibacterial activity. In *Ulva lactuca* methanol extract showed maximum activity against *Pseudomnas aeruginosa* (14 mm), *Staphylococcus aereus (*12 mm) and minimum activity against *Enterobacter aerogens (*11 mm), and *Streptococcus faecalis* (11 mm). Whereas no activity on pathogen like *Bacillus cereus*. (Table: 1, Fig: 1). The *Ulva lactuca* extract used in chloroform showed maximum activity against *Pseudomnas aeruginosa* (13 mm), *Staphylococcus aereus (*13 mm), *Streptococcus faecalis (*12 mm), and minimum activity against *Enterobacter aerogens (*09 mm) and *Bacillus cereus* (07mm). (Table: 1, Fig: 2). The *Ulva lactuca* extract by the way of n- butyl alcohol observed maximum activity against *Enterobacter aerogens (*15 mm) and *Bacillus cereus* (15mm), *Pseudomnas aeruginosa* (13 mm), *Streptococcus faecalis (*12 mm), and minimum activity against *Staphylococcus aereus (*08 mm).In all the three extracts the antibacterial activity increased with increasing concentration. (Table: 1, Fig: 3)

Fig: 1. Antibacterial activity of methanol crude extracts of *Ulva lactuca* against tested pathogens at different concentrations:



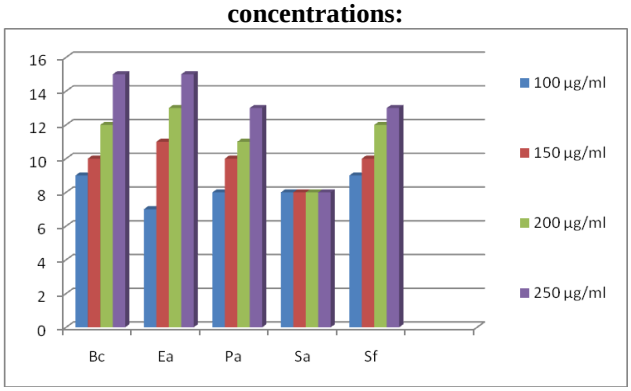
Bc-Bacillus cereus , *Ea- Enterobacter aerogens*, Pa-*Pseudomnas aeruginosa*, Sa- *Staphylococcus aereus* and Sf-*Streptococcus faecalis*,

Fig: 2. Antibacterial activity of chloroform crude extracts of *Ulva lactuca* against tested pathogens at different concentrations:



Bc-Bacillus cereus, *Ea- Enterobacter aerogens*, Pa-*Pseudomnas aeruginosa*, Sa-*Staphylococcus aereus* and Sf-*Streptococcus faecalis*,

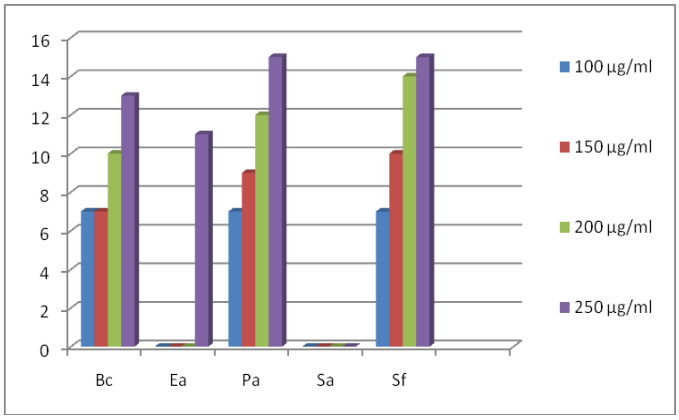
Fig: 3 Antibacterial activity of n-butyl alcohol crude extracts of *Ulva lactuca* against tested pathogens at different concentrations:



µ1000

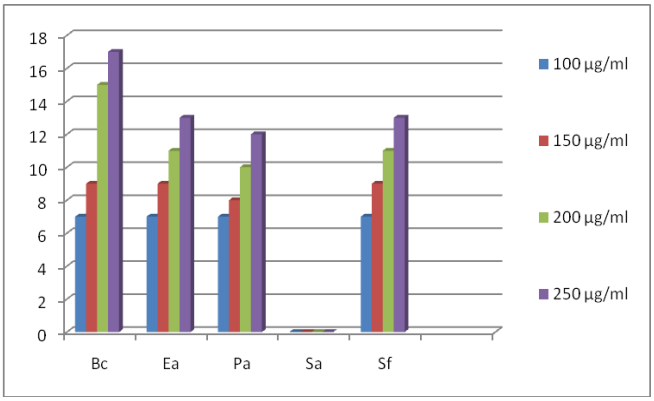
Bc-Bacillus cereus , *Ea- Enterobacter aerogens*, Pa-*Pseudomnas aeruginosa*, Sa- *Staphylococcus aereus* and Sf-*Streptococcus faecalis*,

Fig: 4 Antibacterial activity of methanol crude extracts of *Hypnea musciformis* against tested pathogens at different concentrations:



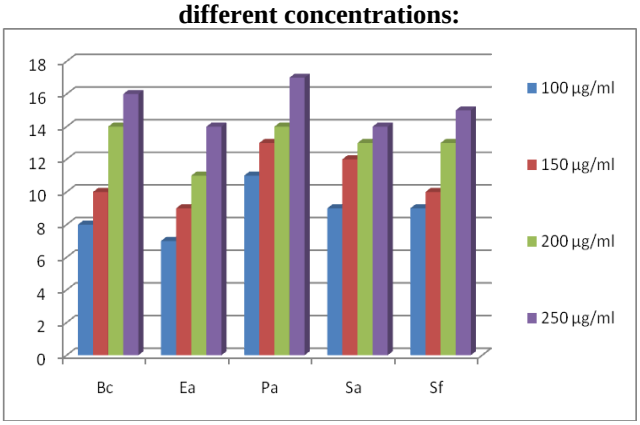
Bc-Bacillus cereus, *Ea- Enterobacter aerogens*, Pa-*Pseudomnas aeruginosa*, Sa-*Staphylococcus aereus* and Sf-*Streptococcus faecalis*

Fig: 5 Antibacterial activity of chloroform crude extracts of *Hypnea musciformis* against tested pathogens at different concentrations:



Bc-Bacillus cereus, *Ea- Enterobacter aerogens*, Pa-*Pseudomnas aeruginosa*, Sa-*Staphylococcus aereus* and Sf-*Streptococcus faecalis*

Fig: 6 Antibacterial activity of n-butyl alcohol crude extracts of Hypnea musciformis against tested pathogens at different concentrations:



Bc-Bacillus cereus, *Ea- Enterobacter aerogens*, Pa-*Pseudomnas aeruginosa*, Sa- *Staphylococcus aereus* and Sf-*Streptococcus faecalis*

Hypnea musciformis

The *Hypnea musciformis* extract obtained using methanol showed maximum activity against *Pseudomnas aeruginosa* (15 mm), *Streptococcus faecalis (*15 mm), *Bacillus cereus* (13mm) and minimum activity against *Enterobacter aerogens (*11 mm). The *Hypnea musciformis* extract showed no activity against *Staphylococcus aereus*. (Table: 2, Fig: 4). The *Hypnea musciformis* extract used in chloroform showed maximum activity against *Bacillus cereus* (17mm) *Enterobacter aerogens (*13 mm) *Streptococcus faecalis (*13 mm), and minimum activity against *Pseudomnas aeruginosa* (12 mm) and no activity against *Staphylococcus aereus*. (Table: 2, Fig: 5). The *Hypnea musciformis* extract by the way of n- butyl alcohol observed maximum activity against all the tested pathogens like *Pseudomnas aeruginosa* (17 mm), *Bacillus cereus* (16mm), *Streptococcus faecalis (*15 mm), *Enterobacter aerogens (*14 mm) and *Staphylococcus aereus (*14 mm). (Table: II, Fig: 4). Like *Ulva lactuca*, the extracts of *Hypnea musciformis* also possessing antibacterial activity and it were increased with increasing concentration.

Discussion

The biodiversity and related chemical diversity of marine ecosystem an infinite reserve of bioactive substances in the field of biomolecules. Due to ecological and biogeographical factors may create disparity in antibiotic content of many algal species [21]. The results obtained from the present study revealed antimicrobial activity by selected solvent extracts such as N-butyl alcoholic extracts of both Ulva lactua and Hypnea musciformis showed highest antibacterial activity against pathogens when compared with standard Amoxicilin. Acetone and ethanol extracts of marine algal *Cladophora facicularis*, *Caulerpa toxifolia, Chaetomorpha antennina, Ulva lactuca* and *Gracilaria corticata* from south east coast of India, showed activity against bacterial [22].

Selvi *et al* [23] screened around 20 algae using methanol and ethanol along Idinthakarai coast and they reported that *Bacillus subtilis* and *Staphylococcus sup*. were highly susceptible to most of the algal extracts. In our experiment Chloroform extracts showed both algal species has maximum antibacterial activity against clinical pathogens. Thirumaran *et al* [24] reported that antibacterial activity of marine macro alga *Caulerpa Scalpeliformis* from Gulf of Mannar coast, the maximum activity was noted in methanol extracts against *Salmonella typhii*, *Micrococcus* sp. and *Shigella boydii*. In the present investigation also methanolic extracts of both algal species showed noticeable antibacterial activity against certain pathogens. Kumaran *et al* [25] studied the susceptibility of *E. coli* in seafood obtained from Cuddalore and Parangipettai fish landing centres against red alga *Kappaphycus alvarezii* and brown alga *Padina boergessenii* extracts as sulfated polysaccharides and polyphenols, respectively.

Seenivasan *et al* [26] studied the antibacterial activity of some marine algae from the southeast coast of India. They reported that acetone, methanol and ethanol extracts of green algae *Ulva fasciata* showed significant antimicrobial activity to *E. coli*. Bansemir *et al*[27] have investigated the antibacterial activities of the extracts from 26 algae species prepared by dichlorometane, methanol and water against five fish-pathogenic bacteria. The highest activities were obtained by the dichloromethane prepared extracts. They have reported that the most active algal species was *Asparagopsis armata* against all tested bacteria. Results from the present screening revealed that the *Ulva lactuca* extract by the way of n- butyl alcohol observed maximum activity against *Enterobacter aerogens*. Sastry and Rao [28] carried out a successive extraction using benzene, chloroform and methanol, and reported the chloroform extract exhibited the strongest activity.

Table: 1. Antibacterial activity of *Ulva lactua* **crude extracts against tested pathogens**

Test Samples	Concentrations µg/ml	Bacterial Pathogens Bc	Ea	Pa	Sa	Sf
Methanol extracts	100	-	07	10	07	07
	150	-	08	12	08	09
	200	-	09	13	10	10
	250	-	11	14	12	11
Chloroform extracts	100	07	-	11	-	07
	150	07	07	12	09	10
	200	07	08	13	11	11
	250	07	09	13	13	12
N-butyl alcohol extracts	100	09	07	08	08	09
	150	10	11	10	08	10
	200	12	13	11	08	12
	250	15	15	13	08	13
Standard (amoxiline)	250	16	14	14	10	15
Control (DMSO)	-	-	-	-	-	-

Zone of inhibition in millimetres, cup diameter: *6mm.* (- No activity)
Bc-Bacillus cereus, Ea- Enterobacter aerogens, Pa- *Pseudomnas aeruginosa,* Sa- *Staphylococcus aereus* and Sf- *Streptococcus faecalis,*

Table: 2. Antibaccterial activity of Hypnea musciformis ***crude*** **extracts against tested pathogens**

Test Samples	Concentrations µg/ml	Bacterial Pathogens Bc	Ea	Pa	Sa	Sf
Methanol extracts	100	07	-	07	-	07
	150	07	-	09	-	10
	200	10	-	12	-	14
	250	13	11	15	-	15
Chloroform extracts	100	07	07	07	-	07
	150	09	09	08	-	09
	200	15	11	10	-	11
	250	17	13	12	-	13
N-butyl alcohol extracts	100	08	07	11	09	09
	150	10	09	13	12	10
	200	14	11	14	13	13
	250	16	14	17	14	15
Standard (amoxiline)	250	16	14	19	15	15
Control (DMSO)	-	-	-	-	-	-

Zone of inhibition in millimetres, cup diameter: *6mm*. (- No activity)
Bc-Bacillus cereus , Ea- Enterobacter aerogens, Pa- *Pseudomnas aeruginosa,* Sa- *Staphylococcus aereus* and Sf- *Streptococcus faecalis,*

It can be seen from the above reports that the efficiency of chloroform in the extraction of seaweeds remains uncertain.

The present study revealed that the N-butyl alcohol extracts of both algal species could be utilized as a good natural source of antimicrobial agents in pharmaceutical industry. The active components responsible for the antibacterial activities need to be evaluated. Further works may be performed on the isolation and identification of the antimicrobial components in *Ulva lactua* and *Hypnea musciformis* for its industrial and pharmaceutical application.

Conflict of interest statement

We declare that we have no conflict of interest.

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