ANTIBACTERIAL ACTIVITY OF MARINE MACRO ALGAE *HYPNEA CERVICORNIS* (RHODOPHYCEAE) COLLECTED FROM CHAGKUMUGAM COASTAL REGION

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Abstract: To evaluate the antibacterial activity of Organic solvent extracts from marine macro algae *Hypnea cervicornis* (Rhodophyceae) against the eight pathogenic bacterial strains. The antibacterial activities of methanol, ethyl acetate and aqueous extracts were tested against various organisms *Eschercia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella sps, Pseudomonous aeruginosa, Spreptococcus sps, Enterobacter sp* and *Neisseria sp.* by using disk diffusion method. The highest antibacterial activity (15.13±0.15mm) was showed by the methanol extract of *Hypnea cervicornis* against *Streptococcus sp* and the lowest activity (7.23±0.25mm) was observed in the methanol extract of *Hypnea cervicornis* against *Proteus vulgaris.* The aqueous extract of *Hypnea cervicornis* was resistant to all bacterial strains.

Keywords: Marine macro algae, antibacterial activity and algal extracts

INTRODUCTION

Marine algae are rich and varied source of bioactive natural products, so it has been studied as potential biocide and pharmaceutical agents [Rangaiah *et al*., 2010]. There have been number of reports of antibacterial activity from marine plants and special attention has been reported for antibacterial and antifungal activities related to marine algae against several pathogens [Kolanjinathan and Stella, 2009]. The antibacterial activity of seaweeds is generally assayed using extracts in various organic solvent for example acetone, methanol-toluene, ether and chloroform-methanol [Cordeiro *et al*., 2006]. Using of organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activity [Tuney *et al*., 2006].

The emergence of multiple drug resistant bacteria has some a major cause for the failure of treatment on infectious diseases [Mathias *et al*., 2000]. As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and some cases all, effective antibiotics [Kapil, 2005]. Much like the situation in human medicine, the use of antibiotics in agriculture, livestock and poultry has accelerated the development of antibiotic resistant strains for complicating treatment to plants, animals and humans [Sorum and Abe-Lund, 2002].

Bacterial infection causes high rate of mortality in human population and aquaculture organisms [Kandhasamy and Arunachalam, 2008]. For example, *Bacillus cereus* is responsible for causing food borne diseases [Wijands, 2008]. *Enterococcus faecalis* is the causative agent of inflammatory bowel disease [Balish and Warner, 2002]. *Pseudomonas aeruginosa* cause disease like mastitis abortion and upper respiratory complications while salmonella sp. causes diarrhoea and typhoid fever [Jawetz *et al*., 1995] *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life threatening illness [Kandhasamy and Arunachalam, 2008]. The antibacterial activity of the seaweed *Gracilaria edulis* associated epiphytic bacteria against human bacterial pathogens from Indian waters and also from west coast of India [Naqvi *et al*., 1981]. Hence, the present work was aimed to study the antibacterial activity of marine macro algae *Hypnea cervicornis* against eight human pathogenic bacteria using methanol, ethyl acetate and aqueous extracts.

MATERIAL AND METHOD

Collection of seaweeds

The macro algae *Hypnea cervicornis* was selected for antimicrobial study. The algae were cleaned with seawater three times and then successively with tap water and distilled water to remove the epiphytes, sand particles and other wastes. Finally, these cleaned fresh materials were cut into small pieces and spread on blotting paper to remove excess water.

Preparation of extract

The samples were shade dried for one month and then pulverized into fine powder using mixer and grinder. The extraction was done by soxhlet extraction techniques. The powdered seaweed samples were extracted using different solvents like aqueous, methanol and ethyl acetate. The samples were cold steeped for 24 h at -18^0C. The samples were then filtered with Whatman No.1 filter paper. The filtrate obtained was evaporated, concentrated and stored (4^0C) in refrigerator for further use.

Test microorganisms

The organisms used for the screening of antibacterial activity are as follows: *Eschercia coli, Staphylococcus aureus, Proteus vulgaris, Klebsiella sps, Pseudomonous aeruginosa, Spreptococcus sps, Enterobacter sps* and *Neisseria sps*. The cultures were collected from inbiotics, Institute of Biology of clinical research, Nagercoil.

Antibacterial Assay

The macro algae extracts were screened against eight human pathogens. Antibacterial activity was carried out using Disc Diffusion Method [Becerro *et al*., and Bauer *et al.*, 1966]. The Antibiotic susceptibility testing by a standardized disk Amikacin was used. Sterile Muller Hinton Agar (Hi-media) plates were prepared and allowed to set. The cultures to be screened were swabbed on top of the solidified media. Discs impregnated with the seaweed extract were placed on the swabbed plate. The plates were incubated at 37^0C for 24 hours. After incubation, the inhibition zone was measured the edge of the Disc to the clear zone in millimeter.

Minimum inhibitory concentration (MIC)

Agar dilution method was used to determine the MICs of extracts against test bacteria [National Committee for Clinical Laboratory Standards, 1997]. The lowest concentration of an extract at which test bacterium did not show any visible growth was taken as its MIC. 1ml of concentration was added to 19ml of MH agar in order to achieve the concentrations [mg/ml]. Plates were dried and divided into sectors based on the number of organisms. Bacterial cultures grown overnight to population density of $10_8 CFUmL^{-1}$ were applied to sectors, each marked for the inoculation of test bacterium. Plates were observed following incubation at 37^0C for 18h. Finally, the number of colony forming units (CFU) of bacteria developed on the agar plates was counted to determine the lowest concentration.

Minimum bactericidal concentration (MBC)

Solutions showing no visible growth of bacteria in MIC assay were pipette out (20µl) from their respective test wells and spread evenly onto a new Muller Hinton Agar (MHA). The plates were incubated for 18 to 24h at 37^0C. Following incubation, the colony forming units (CFU) of the bacteria were counted. MBC is defined as the concentration which results in 99.9% reduction in mg/ml of the original bacterial inoculums, which also means that MBC only allow 0.1% of the bacteria to survive when re-cultured onto the new MHA [Taylor, *et al*., 1983]. Hence, the number of bacteria colony corresponding to 0.1% of the original bacterial inoculums in broth micro dilution test was calculated, where 0.1% is approximately 100cfu. The highest dilution (lowest concentration) of seaweed extract showing number of bacteria colony on the MHA was taken as the MBC value expressed in mg/ml.

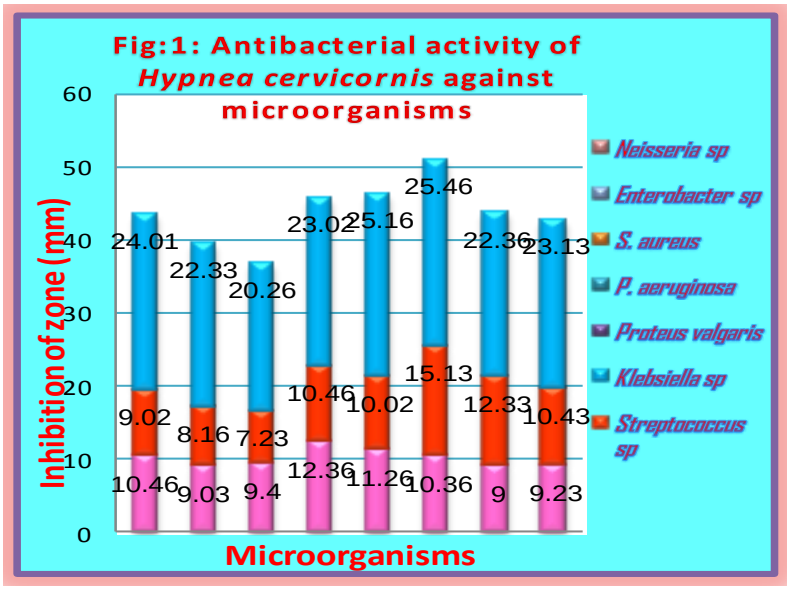
RESULTS

The results on antibacterial activity of *Hypnea cervicornis* against bacterial pathogens were presented in Table-1 and Fig-1. The ethyl acetate extract showed a maximum activity against pathogen like *Klebsiella sp.* 12.36±0.40, *Pseudomonas aeruginosa* 11.26±0.25 and minimum activity against *Enterobacter sp.* 9.00±0.40. The methanol extract showed a maximum activity against *Sterptococcus sp.*15.13±0.15, *Enterobacter sp.* 12.33±0.28 and minimum activity against *Proteus vulgaris* 7.23±0.25. The standard antibiotic Amikacin showed highest activity against the pathogen *Sterptococcus sp.* 25.46±0.41, *Pseudomonas aeruginosa* 25.16±0.15, *E.coli* 24.01±0.10 and lowest activity against *Proteus vulgaris* 20.26±0.25.

Table 1: Antibacterial activity of *Hypnea cervicornis* against bacterial pathogens

Pathogens	Zone of Inhibition (mm)			
	Ethylacetate	Methanol	Aqueous	Antibiotic Amikacin
E.coli	10.46±0.45	9.02±0.20	-	24.01±0.10
Staphylococus aureus	9.03±0.30	8.16±0.20	-	22.33±0.30
Proteus vulgaris	9.40±0.36	7.23±0.25	-	20.26±0.25
Klebsiella sp	12.36±0.40	10.46±0.41	-	23.02±0.26
Pseudomonas aeruginosa	11.26±0.25	10.02±0.20	-	25.16±0.15
Sterptococcus sp	10.36±0.47	15.13±0.15	-	25.46±041
Enterobacter sp	9.00±0.40	12.33±0.28	-	22.36±0.35
Neisseria sp	9.23±025	10.43±0.37	-	23.13±0.15

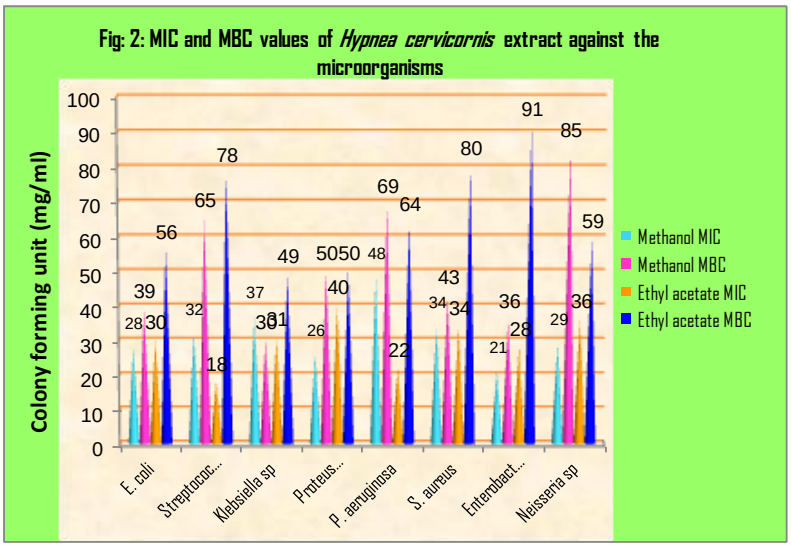
Note: (-) no activity



The results on MIC and MBC (mg/ml) values of *Hypnea cervicornis* extract against microorganisms were presented in Table-2 and Fig-2. The methanol extract of *Hypnea cervicornis* showed highest MIC (48±0.95) against the pathogen *Pseudomonas aeruginosa* and lowest colony forming unit (21±0.81) against the bacteria *Enterobacter sp*. The MBC value of *Hypnea cervicornis* showed highest colony forming unit (85±0.95) against the bacteria *Neisseria sp* and lowest colony forming unit (30±0.83) against the bacteria *Klebsiella sp*.

Table 2: MIC and MBC values of *Hypnea cervicornis* extract against microorganisms

Micro organisms	Methanol		Ethyl acetate	
	MIC	MBC	MIC	MBC
E.coli	28±0.89	39±0.83	30±0.81	56±0.57
Streptococcus sp	32±0.57	65±0.81	18±0.50	78± 0.83
Klebsiella sp	37±0.54	30±0.83	31±0.57	49±0.70
Proteus vulgaris	26±0.57	50±0.70	40±0.81	50±0.83
Pseudomonas aeruginosa	48±0.95	69±0.54	22±0.83	64±0.54
Staphylococcus aureus	34±0.70	43±0.89	34±0.66	80±0.89
Enterobacter sp	21±0.81	36±0.83	28±0.57	91±0.95
Neisseria sp	29±0.56	85±0.95	36±0.95	59±0.89



The ethyl acetate extract of *Hypnea cervicornis* showed highest MIC (40±0.81) against the pathogen *Proteus vulgaris* and lowest colony forming unit (18±0.50) against the bacteria *Streptococcus sp*. The MBC value of *Hypnea cervicornis* showed highest colony forming unit (91±0.95) against the bacteria *Enterobacter sp* and lowest colony forming unit (50±0.83) against the bacteria *Proteus vulgaris.*

DISCUSSION

Seaweeds are used by coastal populations for thousands of years owing to their high nutritional values [Zemke- White and Ohno 1999]. However, the industrialization of seaweeds does not necessarily need their consumption. Medical and pharmaceutical industries are also interested since marine plants are rich in active molecules [Madhusudan, *et al*., 2011]. Indeed, the therapeutic potentials of certain substances are extremely promising, especially as antimicrobial and antiviral factors [Nakajima, *et al*., 2009].

Priyadharshini, *et al*. [2012] reported that the extracts of the *Ulva fasciata* maximum zone of inhibition was observed against *V.alginolyticus* and minimum in *Enterobactor sp*. Mohamed Elanwar *et al*. [2010] documented that methanol extracts of *Ulva faciata* showed highest inhibition zone and ethanol extract of *Sargassum vulgare, Ulva lactuca* showed lowest activity. The antibiotic Fusidic acid and Erythromycin showed maximum activity against *E.aerogenes* and minimum activity was observed against *E.coli* and *S.aureus*. The antibiotic disc penicillin showed maximum activity against *K.pneumoniae* [Emmanuel Joshua Jebasingh, *et al*., 2011].

Prasanna Latha and Hema Latha [2011] documented that the chloroform extract of *Enteromorpha* showed good antibacterial activity when compared to the chloroform extract of *Chaetomorpha*. The chloroform extract of *Enteromorpha compressa* showed the antibacterial activity against all the pathogens compared with the standard antibiotic benzyl penicillin. Adaikalaraj *et al*. [2012] reported that the highest antibacterial activity was shown by the aqueous extract of *Gracilaria verrucosa* against *Pseudomonas aeruginosa* and the lowest activity was observed in the methanol extract of *E.prolifera* against *E.coli*. The microbial stains *Salmonella typhi, Staphylococcus aureus, Bacillus subtilis* and *candida albicans* were showed no antibacterial activity aqueous extract of all seaweeds.

MIC of petroleum ether extract *A. scholaris* more effective than other extracts [Dash and Murthy, 2011]. The lowest MIC (2.0mg/ml) with zone of inhibiting were observed in *E.coli, S.aureus* and *S.aureusR* respectively and the MIC (3.0mg/ml) with zone of inhibitions was observed against *P.aeruginosa, S. typhi* and *P.aeruginosaR* [Singh, *et al*., 2009]. The maximum MIC value was recorded in *C. racemosa* and *P. gymnospora* and *S.dentifolium* which the most effective seaweeds and minimum activities were recorded in *A.fragils* [Selem, *et al*., 2011].

In the present study, maximum zone of inhibition was noted for the red algae *Hypnea cervicornis* against the bacteria *Streptococcus sp.* The methanol extract of *Hypnea cervicornis* showed highest inhibition zone against the pathogen *Klebsiella sp* and ethyl acetate extract of *Hypnea cervicornis* showed lowest activity in *Enterobacter sp.* The same trend was observed in the work carried out by Mohamed Elanwar *et al*. [2010]. The extract of *Hypnea cervicornis* showed highest MBC against the pathogen *Enterobacter sp* and lowest MBC *Proteus valgaris.* The same trend was observed in the work carried out by Salem *et al*. [2011].

CONCLUSION

The results obtained from this study, considering the antibacterial activity guided assay of different solvent extracts from the marine macro algae *Hypnea cervicornis* clearly revealed significant antibacterial activity against all the tested human pathogenic bacteria and it should be thoroughly being investigated for natural antibiotic properties.

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