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Research Article

BIOPOTENTIAL OF SELECTED MACROALGAE FROM KILAKARAI COASTAL REGION IN GULF OF MANNAR, TAMIL NADU, INDIA

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ABSTRACT

Marine macroalgae are the extraordinary source of bioactive substances. In the present research, we used four macroalgae (i.e., *Sargassum wightii, Sargassum muticum, Ulva lactuca* and *Ulva fasciata*), extracted in five solvents (i.e., Chloroform, methanol, ethanol, acetone and butanol) and tested for their antimicrobial activity against five different human pathogens (i.e., *Staphylococcus aureus, Escherichia coli, Bacillus subtilus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*). All the extracts, in particular, brown macroalgae (*S. wightii*) extracted in Butanol, Chloroform and Acetone exhibited the significant level of antimicrobial and larvicidal activity respectively. In particularly, *S. wightii* has an excellent source of larvicidal activity against *Aedes aegypti* and *Culex quinquefaciatus* significantly. This study established acetone extracts of *S. wightii* (brown macroalgae) were highly effective, against bacteria. In future, investigation may help to recognize the bioactive compounds from the brown algae species.

Keywords: Antimicrobial assay, Larvicidal activity, *S. wightii*, Bioactive compound, Gulf of Mannar.

INTRODUCTION

Marine macroalgae are primitive type of plants, growing abundantly in the shallow waters of sea and are the extraordinary sustainable resources in the marine ecosystem, which have been used as a source of food, feed, and medicine (Dhargalkar *et al*., 2016). Macroalgae are used as ornamental plant, but it is also proved to contain medicinal value like antibacterial activity and anti-inflammatory activity (Erdemoglu *et al*., 2003) and also marine macroalgae are have the excellent source of bioactive compounds (i.e., carotenoids, protein, dietary fibre, essential fatty acids, vitamins and minerals (Iliopoulou *et al*., 2002; Metzger *et al*., 2002; Sanchez Machado *et al*., 2002; Viron *et al*., 2000). The brown macroalgae have most effective antioxidants activity, because of the presence of phenolic compounds (Nagai & Yukimoto, 2003) and also play an important role in various diseases (Kohen & Nyska, 2002). Many metabolites isolated from marine algae have been shown to possess bioactive efforts (Khan *et al*., 2008; Oh *et al*., 2008) and (Redon *et al*., 2006). Seaweeds have recently received significant attention for their potential as natural antioxidants. Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world. The Biomolecules from macroalgae were found to be active against human bacterial pathogens (Chellaram *et al*., 2015; Kolanjinathan *et al*., 2009). Based on these backdrops, the present study was investigating the antimicrobial potential activities of five different solvent extracts of four macroalgae from Keelakarai, against five different human pathogens. And highly potential active samples were further examined in larvicidal activity against *A. aegypti* and *C. quinquefaciatus*.

MATERIALS AND METHODS

Sample collection

In the present study, Phaeophyceae (i.e., *Sargassum wightii* and *Sargassum muticum*), Chlorophyceae (i.e., *Ulva fasciata* and *Ulva lactuca*) were collected from low tide

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region in (Latitude: 9° 14' 00" Longitude: 78° 47' 00") kilakarai, Gulf of Mannar, India. All collected macroalgae samples were immediately rinsed with water to remove all kinds of epiphytes and other impurities matters (i.e., sand, mollusks, verm and seagrass). The cleaned samples were immediately kept in sterilized Ziploc bags and transferred to laboratory for further experiments. Algae were identified with the help of morphological key characters and identification manual (Rohit *et al*., 2012).

Extract preparation

The cleaned macroalgae were allowed to shade dry (up to 7 days) and all individual samples were made powder form using mixer grander/ stainless steel pulverizer. The powdered sample of each species (10 gm) was suspended in selective solvent system (i.e., Chloroform, Methanol, Ethanol, Acetone and Butanol) (Merck AR Grade) and kept in a borosilicate soxhlet apparatus for eight hours and until complete extract sample (i.e., until residue solution changed as colourless). After that, extraction of samples was filtered using Whatman No. 1 filter paper. The filtered sample was individually centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected in a separate flask. At each centrifugation, the supernatant was pooled and kept separately. Then the extract was concentrated using a rotary vacuum evaporator (Puchi RII, Switzerland) at 40°C. The final concentrated crude extract was individually stored in sterile air tight bottles and kept in a refrigerator until further use (Celikler *et al.*, 2009).

Antibacterial assay

The crude extract samples (i.e., *S. wightii* and *S. muticum*, *U. fasciata* and *U. lactuca*) with different solvent system (i.e., Chloroform, Methanol, Ethanol, Acetone and Butanol) were examine in antimicrobial activity against human pathogenic (i.e., *Staphylococcus aureus, Escherichia coli, Bacillus subtilus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*) using the well diffusion petri dishes in method. All strains were received from Department of Microbiology, Madurai Medical College, Madurai, Tamil Nadu. The sterile well were individually filled/poured with crude extract of each algal sample (20µL). Agar plates were inoculated uniformly from the broth culture of tested microorganisms. The well was placed on the medium suitable spaced apart and the plates were incubated at 37°C for 24 hours. The growth inhibition radiances caused by the crude extract of each algal species were examined. All tests were performed in duplicate, and the clear radiances (zone formation) (Lima Filho *et al*., 2002).

Larvicidal activity

The bioassay was carried out by the method sholte *et al.* (2003) with minor modifications using different concentration of Chloroform, methanol, ethanol and acetone extract of macroalgae *S. wightii*, against the third instar larvae *Aedes aegypti* and *Culex quinquefaciatus*. For the bioassay, a total of 25 third instar larvae were transferred into 250 mL glass beaker containing different concentration (100µL to 500µL) covered with a mosquito net. Each five replication were maintained for each concentration. The set up was maintained at 27 ± 2°C and 77 ± 4% RH. The mortality of mosquito larvae was noted at 24 hours intervals control and a separately set up was maintained simultaneously treatment.

RESULTS

The present results show that, the highest zone of inhibition was observed in chloroform extract macroalgae (i.e., *S. wightii* and *U. faciata*) are 21 mm and 20 mm with respect to *P. aeruginosa* and *B. subtilis.* Whereas methanol extract of *S. wightii* showed maximum of 16 mm of zone of inhibition against *S. aureus*. The antibacterial efficiency of the ethanol extract (18mm) was comparatively higher to methanol and below to the chloroform extracts (Figure 1). The acetone extract showed a similar antibacterial (20mm) effect to that of the chloroform extract. Among the extract tested, butanol extract expressed an extraordinary antibacterial effect having the zone of inhibition of 22 mm in *S.wightii* against *E. coli*. Besides, they had a wide range of activity against the selected bacterial human pathogens except *K. pneumonia.*

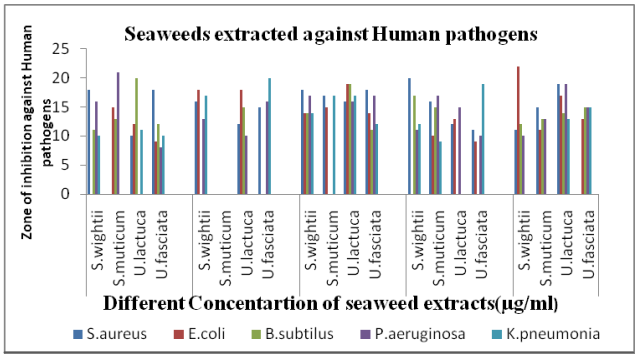

Figure 1. Antimicrobial activity of selected macroalgae against human pathogens.

Based on the antimicrobial studied we are taken only *S. wightii* sample for further experiments. Because of they have potential active against different solvent systems. For in that reason *S. wightii* extract further examine for Larvicidal activity. The table 1 and 2, representing the mortality percentage of solvent extracts of the macroalgae *S. wightii* against III instar larvae of *A. aegypti* and *C. quinquefaciatus*. The bioassay studies exemplified that, acetone extract (162.233µL) showed lowest LC_{50} value followed by chloroform (186.987µL), ethanol (310.87µL) and methanol (345.142µL) against *A. aegypti*. Whereas *Cu. quinquefaciatus*, it was 121.792µL (acetone), 142.867µL (chloroform), 202.688µL (ethanol) and 518.672µL (methanol). Beside, treatments showed the positive slope. Similarly, the chi-square values were also found significant.

Table 1. LC_{50} (24hr) determination for *S. wightii* crude extract influenced toxicityin II instars *A. aegypti* (day concentration/probit regression analysis).

Exposure Period 24hr	LC_{50} ppm	Regression Equation (y=a + bx)	Correlation (r)	95% Fiducially limits		Confidence Limit (fLC_{50})	Chi-Square Test	
				L	U		X^2	Cr
Chloroform	18	Y=7.977+0.186x	0.989	16	21	1.154	24.201	0.004
Petroleum ether	34	Y=1.644+0.049x	0.980	82	82	1.624	4.701*	0.099
Acetone	16	Y=3.58+0.181x	0.964	14	18	1.126	16.768*	0.052
Methanol	31	Y=4.845+0.184x	0.985	27	26	1.140	19.543*	0.021

* = p<0.005 at significant level Chi-Square Test.

Table 2. LC_{50} (24 hr) determination for *S.wightii* crude extract influence toxicity in II instars *C. quinquefasciatus* (Day concentration/probit regression analysis).

Exposure Period 24hr	LC_{50} ppm	Regression Equation (y=a + bx)	Correlation (r)	95% Fiducially limits		Confidence Limit (fLC_{50})	Chi-Square Test	
				L	U		X^2	Cr
Chloroform	14	Y=9.478+0.218x	0.887	12	15	1.132	24.234*	0.004
Petroleum ether	51	Y=2.922+0.09x	0.937	69	82	1.215	15.526*	0.077(NS
Acetone	12	Y=17.79+0.200x	0.882	14	18	1.194	37.665*	0.00
Methanol	20	Y=2.374+0.191x	0.994	24	26	1.199	36.458*	0.00

* = p<0.005 at significant level Chi-Square Test.

DISCUSSION

Many studies were reported on biological activities of algal extracts from different coastal regions around the world (Harada *et al*., 1997). Seaweeds are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry (Eluvakkal *et al*., 2010). Basha & Muthukumar, 2014 Reported alkaloids play important role defense mechanisms against pathogenic organism and herbivores. The present investigation has studied the antimicrobial activity of different solvent extracts of macroalgae. Generally, presence or absences of different chemical constituents in macroalgae extracts were responsible for different biological activities. Saponin possesses specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include antimicrobial, anti-inflammatory, antifeedent, and hemolytic effects (Francis *et al*., 2002; Xu *et al*., 1996). Similarly, flavonoids, the major group of phenolic compounds reported for their antimicrobial, antiviral and spasmolytic activity (D'souza *et al*., 2007).

The acetone extracts and chloroform extracts also unveiled good antibacterial activity against tested bacterial human pathogens (Jebasingh *et al*., 2011). Proved that *S. wightii, U. fasciata, C. racemosa* and *P. gymnospora* extracted in different solvents (chloroform, methanol, petroleum ether, acetone and butanol) and tested against human pathogens like *S. aureus, S. mutants, B. subtilus, P. aeruginosa, K. pneumoniae, E.coli* and *S. typhimurium* shows maximum inhibitory activities. Antimicrobial assay revealed that, butanol extract showed higher antibacterial potential followed by chloroform and acetone extract. Manilal *et al.*, 2010 Studied the antimicrobial potential of marine organisms collected from the southwest coast of India. The antibacterial effect of the crude extracts and purified fractions of *Cladophora glomerata* against human pathogen was investigated by Yuvaraj *et al*. 2011. Similarly, the present investigation has exemplified that, the all the tested extracts showed higher antibacterial activity towards gram positive bacterial species. It was due to the more complex structure of the gram negative bacteria (Chakraborty *et al*., 2010; Pesando & Caram, 1984; Reichelt & Borowitzka, 1984) possessing peptidoglycon containing n-acetyl glucosamine and n-acetyl muramic acid.

The seaweeds have been also explored to larvicidal activity against different vector species. The marine plant extracts contain promising agent against larvicidal activity (Manilal *et al*., 2011; Selvin *et al*., 2004) had evaluated the methanol extract of the brown algae, *Lobophora variegata* against mosquito pupae, nematodes and plant seeds. Similarly, Beula *et al*. 2011 studied the larvicidal effects of macroalgae i.e., *Enteromorpha intestinalis*, *Dictyota dichotoma* and *Acanthopora spicifera* against 3rd instar larvae of *Aedes aegypti*. Investigation revealed

that ethanol and chloroform extracts of *U. lactuca* recorded the highest percentage of larval mortality in *Spodoptera littoralis* larvae (36.66 and 23.33 %, respectively) at 25 mg/ml. Thus the present study has demonstrated the potential antibacterial and larvicidal agent from non commercial eco-friendly sources. The study would be further elevated to elute the potential antibacterial fraction from seaweed extracts as novel antimicrobial as well as eco-friendly non-chemical larvicidal agent to curb the prevalence of bacterial as well as vector borne diseases.

Further research studies are being carried out on the other species seaweeds from the same habitat in order to provide complete data of the antimicrobial potential seaweeds of kilakarai. It is also necessary for successful separation, purification and characterization of biologically active compounds using chromatographic and spectroscopic techniques for the synthesis novel antibiotics.

CONCLUSION

The present study demonstrated that *S.wightii* has potential of antimicrobial activity against different human pathogens. And also have an excellent source of Larvicidal activity in *A. aegypti* and *C. quinquefasciatus* significantly. These findings were recommended that *S.wightii* could be a potential source of antimicrobial and Larvicidal activity. In Further purification, identification and characterization of the active compounds would be our priority in future studies.

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