ISSN 0975-6299 Vol 3/Issue 2/April – June 2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE MARINE BIOTECHNOLOGY

EFFECT OF SECONDARY METABOLITES OF THE SEAWEED (*HALIMEDA MICRONESIA*) AT LAKSHADWEEP ISLANDS AGAINST AQUATIC PATHOGENS

R.GANESHAMURTHY [1]*, T.T.AJITH KUMAR [2] AND N.B.DHAYANITHI [3]

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India

R.GANESHAMURTHY
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India

ABSTRACT

Marine ornamental fishes are remarkably valuable due to their high demand in domestic and international markets. It is a growing global interest to maintain these fishes in captivity. The bacterial and fungal infection is the major drawbacks in captive condition. In the present study, in-vitro screening of organic solvent extracts (methanol and acetone) of green algae *Halimeda micronesia* were tested against the aquatic pathogens *Providencia rettgeri, Aeromonas hydrophila, Vibrio alginoticus, V. parahaemolyticus, Proteus proteus, Pseudomonas flurescens, Entrobacter* sp*, Flavobacterium* sp*, Edwarsiella tarda,* and *micrococcus* sp. The antibacterial activity assay was tested using agar well diffusion method and the zone of inhibition was compared with the same produced by the antibiotic (Chloramphenicol) as a positive control. Crude extracts were analyzed with Fourier transform infrared spectroscopy (FT-IR) for identifying functional group of the crude extract.

KEY WORDS

Seaweed, *Halimeda micronesia*, Fish pathogen, Antibacterial activity, FT-IR

INTRODUCTION

It has been estimated that 1.5 to 2 million people worldwide keep marine aquaria with 600,000 households in the United States alone [8]. Estimated value of marine ornamental trade is 200- 330 million US$ per year [2]. Bacterial diseases are responsible for heavy mortality in marine ornamental fishes. The problems in the culture systems are usually tackled by preventing disease outbreaks or by treating the infection with antibiotics or chemicals [12,16].

Seaweed is a renewable living resources found in the coastal water bodies. Seaweed are known for their nutrient and chemical composition and it serves as an important source of bioactive natural substances. They have been used as food in the Asia countries for centuries as it contains carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals [13,17]. Biostimulant properties of seaweeds are explored for the use in agriculture and there antimicrobial activities for the development of novel antibiotics. Among the different compounds with functional properties found in seaweed antioxidants are the most widely studied [9] . In the present study, antibacterial activities of different solvent extracts of *Halimeda micronesia* were investigated against marine ornamental fish pathogens [3].

MATERIALS AND METHODS

Collection of samples

Fresh seaweed, *Halimeda micronesia* were collected from inter tidal at Agatti Island (10°51´N and 72°11´E), Lakshadweep, India. They washed thoroughly using seawater to remove extraneous materials and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were shade dried and ground to powder using mortar and pestle. The powdered sample was subsequently stored for further studies

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Solvent Extraction

The finely powdered samples as weighed at 30 g and dissolved in 100 ml of solvents (Methanol and Acetone) and were placed it on a shaker at 120 rpm for 24 hours at 35^0 C to allow full extraction of the active compounds. After 24 hours, material was filtered using Whatman filter paper (No 1) filter with a Buchner funnel using suction pressure followed by centrifugation at 5000 rpm for 20 min. The supernatant were filtered and crude extracts stored at refrigerator until further use [9].

Test microorganisms

The bacterial fish pathogens such as *Providencia rettgeri, Aeromonas hydrophila, Vibrio alginoticus, V. parahaemolyticus, Proteus proteus, Pseudomonas flurescens, Entrobacter sp, Flavobacterium sp, Edwarsiella tarda* and *Micrococcus sp* were obtained from the marine ornamental fish hatchery, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Tamil Nadu, India.

Antibacterial assay

The agar well diffusion method was followed for antibacterial susceptibility. The ten different pathogens were inoculated a muller hinton agar. The 6mm well were impregnated with gel puncture and were added to the well 100 µl of the extracts. The inoculated plates

were incubated for 24-28 hrs at 35^0 C and the inhibition zones were measured around the well (mm diameter).Positive control was maintained with Chloramphenicol and negative control solvent alone [5].

FTIR analysis

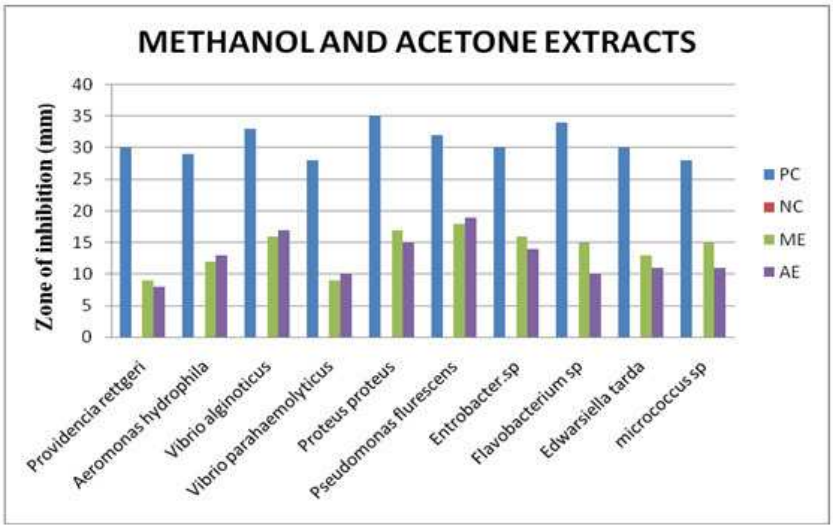
Infrared reflectance vibrational spectra were carried out on powdered glass samples using a spectrometer with instrument resolution of about (1 cm−1), in the wave number region (4000–400 cm−1) at room temperature [11].

RESULT DISCUSSION

Results obtained in the present study confirmed that the tested *Halimeda micronesia* extracts possess antibacterial activity against *Providencia rettgeri, Aeromonas hydrophila, Vibrio alginoticus, V. parahaemolyticus, Proteus proteus, Pseudomonas flurescens, Entrobacter sp, Flavobacterium sp, Edwarsiella tarda* (Fig 1). The crude extracts of *Halimeda micronesia* had the most potent inhibitory activity against *Vibrio alginoticus, Proteus proteus, Pseudomonas flurescens* and *Enterobacter*. However, *Providencia rettgeri, Aeromonas hydrophila, Vibrio parahaemolyticus, Flavobacterium* sp, *Edwarsiella tarda* and *Micrococcus* sp had significantly lower antimicrobial activity in acetone and methanol extracts of *Halimeda Micronesi*. The antibacterial activity can be ascribed due to the presence of secondary metabolites.

Figure 1

Antibacterial activity of solvent extract of Halimeda micronesia against bacterial strains



PC= Positive control, NC= Negative control, Me = Methanol Extracts, AE= Acetone Extracts

The acetone and methanol extracts of *Halimeda micronesia* were subjected to FT-IR analysis. Based on the results, the functional groups associated in the extracts were determined. The absorption bands associated with CH_2 and CH_3 aliphatic compounds formation occurred between 2850, 1458 and 1376cm^{-1}. The regions from 1734 to 1638 cm−1 represented C=O in anhydrides and C=O secondary amides, in that order. The C—N band of aromatic amines appeared between 1237 cm−1. The SO_3H in solfonic acids, C — NH_2 in primary aliphatic amines, Ar — OH in phenol are those bands of esters that

appeared approximately at the range of 1610, 1110 and 669 cm−1.The bands related to P—H were stretched to wave number as 2344 cm−1 which showed enhancement when compared to that of pure butter spectra of phosphines (Table 1 and Fig 2, 3).

Table 1
Functional Group and Class of compounds absorb in the infrared wavenumber

S.No	Range(cm^{-1})	Group and Class	Assignment
1	2850(ms)	CH3 and CH2 aliphatic compounds	= C—H stretch
2	2344(m)	— PH in phosphines	P—H stretch; sharp peak
3	1734(vs)	C=O in anhydrides	C=O sym stretch; part of doublet
4	1638(vs)	C=O secondary amides	C=O stretch(Amide I band)
5	1458(vs)	CH3 in aliphatic compounds	CH3 antisym deformation
6	1376(s)	CH3 in aliphatic compounds	CH3 antisym deformation
7	1237(s)	C—N in aromatic amines	C—N stretch
8	1161(vs)	SO3H in solfonic acids	S=O stretch
9	1110(s)	C — NH2 in primary aliphatic amines	C—N stretch
10	669(s,br)	Ar — OH in phenol	OH out of plane deformation

Figure 2
FTIR Spectrum of Acetone Extracts

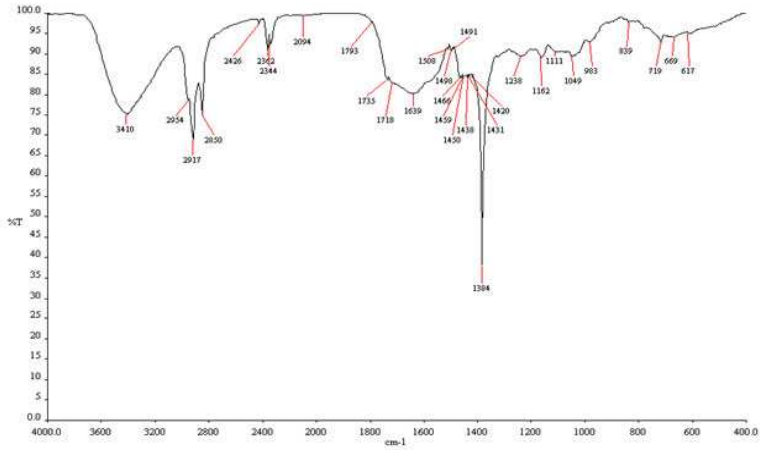
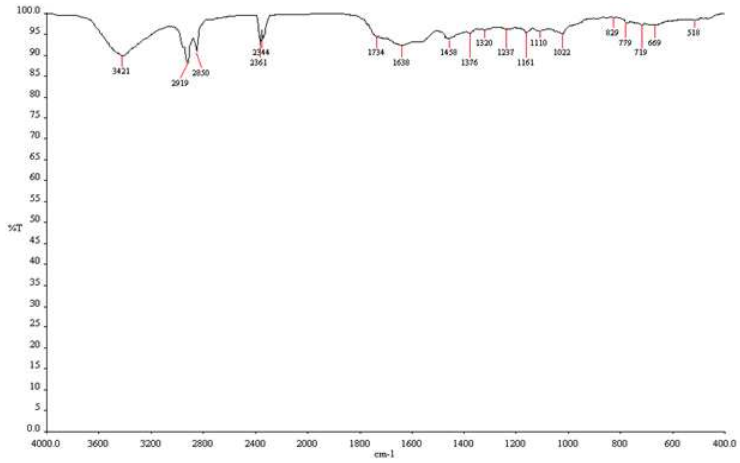


Figure 3
FTIR Spectrum of Methanol Extracts



Marine organisms are a rich source of novel and biologically active compounds. Primary or Secondary metabolites produced by these organisms may be potential biologically active metabolites of importance in the pharmaceutical industry. To date, number of chemically unique metabolites has been isolated from marine origin and some of them are under study to develop new pharmaceuticals. The cell extracts and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria [18].

Ishibashi et al. (1993) [20] and Wu et al. (1997) [21] reported that seaweeds such *Aglaia elliptifolia* and *Aglaia odorata* showed insecticidal and cytotoxic activities. In this study the extracts of *Halimeda micronesia* were found to encompass antibacterial activity against pathogens like *V. alginolyticus* (16mm), *E. tarda* (13mm) and *A. hydrophila* (11mm) which confirms that sea weeds in general can posses active biological compounds.

Crasta et al (1997) [15] studied the inhibitory activity of various marine algae like *Cladophora fascicularis*, *Caulerpa taxifolia*, *Chaetomorpha antennina*, *Ulva lactuca* and *G. corticata* which were collected from south-west coast of India in three seasons. The Acetone and ethanol extracts of marina algae used in their study showed significant inhibitory activity. But the inhibitory activity varied from season to season. Our present study showed that the sensitivity of gram negative bacteria pathogens is higher in acetone extracts of *Halimeda micronesia* which showed potent inhibitory activity against *Vibrio alginoticus, Proteus proteus, Pseudomonas flurescens and Entrobacter* sp. But the acetone extracts exhibited moderate sensitivity against *Providencia rettgeri, Vibrio parahaemolyticus, Flavobacterium* sp, *Aeromornas hydrophila and Edwarsiella tarda*. Similarly, Abdul Kader Mydeen (2010) [1] stated that extracts of *S. torvum* fruit coat exhibited significant antibacterial activity against *A. hydrophila* and produced zone of inhibition of 11mm

The Methanol and Acetone extracts of *Halimeda Micronesia* seaweeds showed maximum inhibition against *Aeromonas hydrophila, Enterobacter sp, Vibrio alginoticus and Vibrio parahaemolyticus* which was similar to the results of Dhayanithi *et al*., (2010) [4]. Their study reported that the ethanol extracts of neem showed inhibitory activity against *Aeromonas hydrophila, Enterobacter sp, Vibrio alginoticus and Vibrio parahaemolyticus*. Choudhury (2005) [3] tested 3 species of marine algae and five species of mangroves for inhibitory activity and found that activity was clearer in some taxonomic groups but it varied seasonally. They found no activity was marked by *Gracilaria* sp., *Enteromorpha* sp. and *Cladophora dalmatica*. But, in our case the acetone and methanol extract of *Halimeda micronesia* showed high and moderate antibacterial activity.

Sastry and Rao (1994) [15] found the benzene extract of *G. corticata* showed antibacterial activity only against *Salmonella typhi* and *Escherichia coli* whereas the methanol and chloroform extracts had activity against *P. aeruginosa*. Comparing the results of with our study, the acetone and methanol extracts of *Halimeda micronesia* showed good activity against *Aeromonas hydrophila, Enterobacter sp, Vibrio alginoticus, Proteus proteus and Vibrio parahaemolyticus*.

Robic et al (2009) [14] predicted the composition of all the data set using the Calibration models, which got established using the leave-one-out criterion. The strong O–H stretching modes were centered on 3,300 cm-1. Ray and Lahaye, Bociek and Welti and Pawan reported infrared spectrum of ulva showed strong absorbances at about 1650, 1250 and 1070 cm-1 and little ones were about 1400, 850 and 790 cm-1. Carboxylate groups showed two bands: an asymmetrical stretching band near 1,650 cm-1 and a weaker symmetric stretching band near 1400 cm-1, and sulfate esters showed a major band about 1250 cm−1. The 1200-1000 cm-1 region is dominated by sugar rin vibrations overlapping with stretching vibrations of (C– OH) side groups and the (C–O–C) glycosidic bonds vibration. In this present study the absorption bands associated with CH_2 and CH_3 aliphatic compounds, C=O in anhydrides and C=O secondary amides, C—N band of aromatic amines, SO_3H in solfonic acids, C — NH_2 in primary aliphatic amines and Ar — OH in phenol are indicated in the acetone and methanol extracts

.

CONCLUSION

The mining of natural products from secondary metabolites will be an important resource in tapping the valuable active compounds. In the present study the antibacterial activity of seaweeds has stated the rich source of bioactive compounds present in the seaweed *Halimeda micronesia* which can

impose defense against harmful organisms. The efficiency of antimicrobial activity of natural products from marine macro algae is said to be higher with organic solvents like acetone and methanol which was confirmed by FTIR analysis. The above findings were well correlated to the present results by their efficiency in antimicrobial effect on ten aquatic pathogenic microorganisms by the acetone and methanolic extracts of *Halimeda micronesia*.

ACKNOWLEDGMENTS

Authors are thankful to Prof. T. Balasubramanian, Dean, Centre of Advanced Study in Marine Biology and the authorities of the Annamalai University for providing facilities and the Centre for Marine Living Resources and Ecology (Ministry of Earth Sciences), Kochi for financial assistance.

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