ANTIBACTERIAL ACTIVITY OF DIFFERENT SEA GRASS EXTRACTS AGAINST SOME HUMAN EYE PATHOGENS

J. Sangeetha¹ and S. Asokan¹*

PG and Research Department of Microbiology, Marudapandiyar College, Thanjavur, South India - 613 403.

ABSTRACT

In this present investigation the antibacterial activity of *Halophila ovalis*, *Cymodocea serrulata* and *Halodule pinifolia* against eye infectious pathogens like *E. coli*, *Enterococcus faecalis*, *Corynebacterium*, *Bacillus subtilis* *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, Methicillin Sensitive *Staphylococcus aureus*, Methicillin Sensitive *Staphylococcus saprophyticus* and Methicillin Sensitive *Staphylococcus epidermidis* were evaluated using different solvent extracts (chloroform, ethyl acetate, ethanol and hexane). Antibacterial activity of these sea grasses showed significant activity in the order of *H. ovalis > H. pinifolia > C. serrulata*.

KEYWORDS: Sea grasses, Eye pathogens, antibacterial activity, *Halophila ovalis*, *Cymodocea serrulata* and *Halodule pinifolia*.

INTRODUCTION

Sea grasses are submerged marine angiosperms growing abundantly in tidal and sub tidal areas of all seas except in the Polar Regions. Sea grass biomass is used as human food especially by coastal populations (Hemminga & Duarte, 2000). In folk medicine, seagrasses have been used for a variety of remedial purposes, like, fever, skin diseases, muscle pains, wounds and stomach problems etc. (de la Torre-Castro & Rönnbäck, 2004). In India, seagrasses were used as medicine (treatment of heart conditions, seasickness), food (nutritious seeds), fertilizer (nutrient rich biomass) and livestock feed (goats and sheep) (Newmaster et al., 2011). Seeds of *Enhalus acoroides* are thought to have aphrodisiac and contraceptive properties (Aliño et al., 1990).
New trends in drug discovery from natural sources emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities. These entities are the source of new lead for treatment of many diseases such as cancer, AIDS, inflammatory condition, arthritis, malaria, viral, bacterial and fungal diseases (Nazar et al., 2009).

Only very few studies are available on antifungal, antibacterial and antiviral activities of crude solvent extracts of the seagrasses. However recent reports on the phytochemical constituents of seagrasses of the Gulf of Mannar, South India are limited except few reports (Athiperumalsami et al., 2008; Ragupathi et al., 2010). There is no finding in the field dealing with antibacterial activity of Tamilnadu coastal Seagrass extracts on eye pathogenic bacteria. So the present study was undertaken to investigate the antibacterial activity of seagrasses, *Halophila ovalis*, *Cymodocea serrulata* and *Halodule pinifolia* collected from intertidal region of the Mandapam coast, Tamil Nadu, India, against some eye bacterial pathogens.

**MATERIALS AND METHODS**

**Sample Collection**

Fresh leaves of *Halophila ovalis*, *Cymodocea serrulata* and *Halodule pinifolia* were collected from the intertidal region of the Mandapam coast (Lat. 09° 17.417’N; Long. 079° 08.558’E) and immediately brought to the laboratory in sterile plastic bags containing water to prevent evaporation. Sea grasses were washed thoroughly with Distilled water to remove extraneous materials and shade-dried for 10 days at room temperature until constant weight obtained. The dried Sea grasses were powdered in an electric mixer and sieved through 0.8 mm² sieve plate and stored in refrigerator for future use.

**Preparation of Sea grass extract**

Sea grass powder were soaked in 2L organic solvents with the increasing order of polarity viz., hexane, ethyl acetate, chloroform and ethanol (1:4 w/v), and kept for 10 days in a shaker. The extraction was repeated thrice, pooled and filtered through Whatmann No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure using a rotary flash evaporator. The dry aqueous extracts were lyophilized and stored in a refrigerator until further analysis.

**Antibacterial susceptibility test**

Eye infected pathogens (*E.Coli*, *Enterococcus faecalis*, *Corynebacterium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Methicillin Sensitive Staphylococcus*...
aureus, Methicillin Sensitive Staphylococcus saprophyticus and Methicillin Sensitive Staphylococcus epidermidis) used in this study were collected from Dr. Agarwal Eye Hospital, Tamilnadu, India.

Antibacterial activity was evaluated using disc diffusion assay on Muller Hinton agar (Norris and Fenical, 1985). Based on this method the sterile filter paper discs 6 mm in diameters (Whatman No.1), were loaded with 100µg, 250µg, 500µg and 1000µg/ml of crude extracts and air-dried. Discs were loaded with 5% DMSO (Dimethyl sulfoxide) was used as negative control and streptomycin and amikacin was used as Standard. The discs were placed on Muller Hinton agar (HiMedia, India) plates inoculated for each treatment for 24 h at 37°C. Zone of inhibition was recorded in millimeters and mean values were reported and it was compared with Standard Chart.

RESULTS
The antibacterial activity of *Halophila ovalis*, *Cymodocea serrulata* and *Halodule pinifolia* extracts on eye pathogens were presented in Figure 1-3. The results showed in the *Halophila ovalis* possess the significant activity against the eye pathogens than *Cymodocea serrulata* and *Halodule pinifolia* in controlling their growth. The results of two ways ANOVA reveal that there is a significant difference (P≤0.01) in antimicrobial activity of all three sea grass extracts.

Among the four solvents (chloroform, ethyl acetate, ethanol) extracts inhibited the growth against *E.Coli, Enterococcus faecalis, Corynebacterium, Bacillus subtilis* of which chloroform extracts were effective than others two. Hexane extract of two seagrasses *Halophila ovalis, and Halodule pinifolia* inhibited the growth against *Pseudomonas aeruginosa, Klebsiella pneumonia*, Methicillin Sensitive *Staphylococcus aureus*, Methicillin Sensitive *Staphylococcus saprophyticus* and Methicillin Sensitive *Staphylococcus epidermidis*. The highest zone of inhibition (15 mm) was recorded in hexane extract of *Halophila ovalis* against *Corynebacterium* followed by (14 mm) with *H. pinifolia* and chloroform extract of *H. pinifolia* against *Corynebacterium*. 
DISCUSSION

There have been a number of reports that demonstrating the antimicrobial activity of seaweeds, mangroves and other marine forms and only limited information were available
from the sea grasses of the corners of the world and even very mere information available from India. The aim of this study is to evaluate and compare the ability of different sea grass extracts to produce bioactive compounds of potential therapeutic interest. Antimicrobial activities found in sea grasses were considered to be an indication of synthesis of bioactive secondary metabolites Rengasamy et al., (2010).

The antibacterial activity of different extracts of *H. ovalis*, *C. serrulata* and *H. pinifolia* on four eye pathogens are effective. Among them chloroform was more effective than the others. Hexane extract of two seagrass *H. ovalis* and *H. pinifolia* inhibited the growth against *E.Coli* and *Corynebacterium*. This showed that chloroform is suitable for extracting active compounds from sea grasses. Earlier reports were suggested that the present investigation like, Alam et al., (1994) who found that methanolic extract of *Enhalus acoroides* were effective against *S. aureus*, *K. pneumoniae* and *P. aeruginosa* than the hexane extract. Sreenath Kumar et al., (2008) reported *Halophila* and *Zostera* were more effective than *Cymodacea*. Balasubramanian et al., (1974) reported that the lipid and water-soluble phenolic extracts of both leaf and root-rhizome fractions of *H. pinifolia* are the most promising of antibacterial activity. Rengasamy et al., (2010) reported that the methanol extract of *H. pinifolia* exhibited strong antibacterial activity against human pathogens. This result is in agreement with the present finding that *H. pinifolia* showed second maximum activity. Rengasamy et al., (2012) reported that the six sea grass and their potential effect on clinically important UTI bacteria. The result indicated that, *C. serrulata* and *H. pinifolia* exhibited predominant growth inhibitory activity against all the UTI bacteria.

Rengasamy et al., (2010) reported that the Hexane extract of the seagrasses did not showed activity against some human pathogens. In the present study thus, Hexane extracts of the seagrasses *H. ovalis* and *H. pinifolia* inhibited the growth against *E.Coli* and *Corynebacterium*.

Kumar et al., (2008) recorded ethyl acetate and methanol extract of sea grasses showed maximum activity against most of the pathogen and suggest ethyl acetate to be the best solvent for isolation of bioactive substance from seagrasses. Rengasamy et al., (2010) confirmed the methanol extract of the test plants showed better antimicrobial activity than the other extracts.
CONCLUSION
In the present study, chloroform, ethyl acetate extract of sea grass showed maximum activity against four pathogens and suggested chloroform to be the best solvent for isolation of bioactive substance from seagrass to development of novel medicinal products against eye infections and this research need to be isolating the specific antibacterial agent against eye infected pathogens using some advanced techniques.

ACKNOWLEDGEMENTS
The authors are grateful to Dr. C. Sreenath Kumar and Dr. Suresh for providing necessary facilities. We also thank Asst. Prof. D. Arvind Prasanth, Faculty of Microbiology, Periyar University, Salem.

REFERENCES


