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Antibacterial potential of three seagrasses against human pathogens

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ABSTRACT

Objective: To evaluate the antibacterial activity of *Halophila stipulacea (H. stipulacea)*, *Cymodocea serrulata (C. serrulata)* and *Halodule pinifolia (H. pinifolia)* against seven human bacterial pathogens. **Methods:** The antibacterial activities of the extracts on the various test organisms using disc diffusion method and Minimum Inhibitory Concentraction (MIC). **Results:** Methanol and chloroform extracts of all the three seagrasses were active against all the tested pathogens, whereas the hexane extract of seagrasses was not active against *Staphylococcus aureus (S. aureus)*. Antibacterial activity of three seagrass screened, was in the order of *H. pinifolia* > *H. stipulacea* > *C. serrulata*. **Conclusions:** This antibacterial studies can further investigated on seagrasses for purification of bioactive substance and its possible utility in disease control.

1. Introduction

Seagrasses are submerged marine angiosperms growing abundantly in tidal and subtidal areas of all seas except in Polar Regions. Though seagrasses comprises few species, their importance to estuarine and coastal marine environments and to pharmaceuticals is significant. Generally seagrasses are rich source of secondary metabolites which is believed to be a defense mechanism to these plants[1]. The use of the roots of *Enhalus acoroides (E. acoroides)* as a remedy against stings of different kinds of rays and scorpion is very popular. *Cymodocea* spp. is used as a tranquillizer for babies, as soothing help during pregnancy and against cough and malaria. *Halophila* spp. is a strong medicine against malaria and skin diseases and found to be very effective in early stages of leprosy. Seventeen and eighteen century Spanish colonial documents suggest that seeds of *Halics* were major food source of Seri[2]. *Halophila ovalis (H. ovalis)* was used by the fishing communities of Cuddalore and Nagapattinam districts of Tamilnadu, South India as medicine to treat various skin diseases, burns and boils[3].

Only very few studies are available on antifungal, antibacterial and antiviral activities of crude solvent extracts of the seagrasses. Marine and estuarine submersed aquatic angiosperms or seagrasses produce antimicrobial compounds that may act to reduce or control microbial growth. Most of the works carried out so far on seagrasses are all in combination with seaweeds and other plants. Naqvi *et al*[4] tested the extracts of seaweeds and seagrass from Indian coasts for antiviral, antibacterial, antifungal, antiprotozoa, antifertility and pharmacological properties. Premanathan *et al*[5] tested the extract of the seaweed, seagrasses and mangroves for their antiviral activity. The methanol and hexane extracts of seagrass *E. acoroides* and some seaweed were tested against *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus(S. aureus)*, *Pseudomonas aeruginosa(P. aeruginosa)* and *Klebsiella pneumoniae*(*K. pneumoniae*)[6]. Praba Devi *et al*[7] tested the extract of 16 marine plants for the anti bactericidal activity against 15 strains of marine fouling bacteria. Bhosale *et al*[8] studied the antifouling potential of some marine organisms against *Bacillus* and *Pseudomonas* species and reported that the seagrass *Cymodocea rotundata* exhibited mild activity against all the bacterial strains. Four new metabolites have been isolated from organic extract of *Cymodocea nodosa (C. nodosa)* collected from the coastal area of

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Porto Germeno, in Attica Greece and evaluated for their antibacterial activity against multi drug resistant pathogens including methicillin-resistant strains of *S. aureus* and rapidly growing mycobacteria, *Mycobacterium phlei (M. phlei)*, *Mycobacterium smegmatis(M. smegmatis)* and *Mycobacterium fortuitum (M. fortuitum)*[9]. The antibacterial properties of three seagrasses namely *Cymodacea serrulata(C. serrulata)*, *H. ovalis* and *Zostera capensis* were tested against human pathogens such as *S. aureus*, *Bacillus cereus (B. cereus)*, *B. subtilis*, *Escherichia coli (E. coli)*, *Salmonella paratyphi (S. paratyphi)*, *Salmonella typhimurium (S. typhimurium)* and *Micrococcus luteus (M. luteus)*, using six different solvent system for extraction namely petroleum ether, chloroform, ethyl acetate, acetone, methanol and water[10].

The present study was undertaken to investigate the antibacterial activity of three seagrasses *Halophila stipulacea(H. stipulacea)* (Forsk.) Asch., *Cymodocea serrulata* (R.Br.) Asch. & Magnus and *Halodule pinifolia (H. pinifolia)* (Miki) Hartog collected from intertidal region of the Mandapam coast, Tamil Nadu, India against seven human bacterial pathogens.

2. Materials and methods

Fresh leaves of *H. stipulacea*, *C. serrulata* and *H. pinifolia* (Miki) Hartog 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. Seagrasses were washed thoroughly with tap water to remove extraneous materials and shade-dried until constant weight obtained and ground in an electric mixer. The powdered samples were then stored in refrigerator for future use.

Seagrass powder were soaked in organic solvents with the increasing order of polarity viz., hexane, chloroform and methanol (1:4 w/v), and kept for two weeks at room temperature, and the extracts were collected and concentrated. The concentrates were reconstituted with their respective extracts (5 mg/mL).

The bacterial strains *S. aureus*, *Vibrio cholerae(V. cholerae)*, *Shigella dysentriae (S. dysentriae)*, *Shigella bodii (S. bodii)*, *S. paratyphi*, *P. aeruginosa* and *K. pneumoniae* used for the present study were received from Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamilnadu, India.

Antibacterial activity was evaluated using the diffusion technique on Muller Hinton agar[11]. Briefly, sterile filter paper discs 6 mm in diameters (Whatman #1), were loaded with 25 μL of different extracts (100 mg/mL) and air-dried. Discs containing solvents alone were used as negative controls and streptomycin was used as a positive control. The discs were placed on Muller Hinton agar (HiMedia, India) plates inoculated with each of the previously mentioned microorganisms. Plates were incubated in triplicate for each treatment for 24 h at 37 ℃. Zone of inhibition was recorded in millimeters and mean values were reported.

Micro dilution method was used to determine the Minimum Inhibitory Concentration (MIC)[12-14]. All tests were performed in Muller-Hinton broth medium (Himedia). About 100 mg/mL of concentration of the extract was dissolved in water+DMSO (2%) and diluted to give serial two-fold dilutions that were added to each medium well. Each well was inoculated with 5 μL of suspension containing 10^8 CFU/mL of bacteria The MIC was determined by measuring optical density of the culture broth in a automatic ELISA tray reader adjusted at 630 nm (Versa Max 4.5). Streptomycin was used as a positive control. The MIC was defined at which concentration of the seagrass extract microbial growth was completely absent.

3. Results

The antibacterial activity of *H. stipulacea*, *C. serrulata* and *H. pinifolia* extracts on seven human pathogens were presented in Figure 1-3. Of the three seagrasses screened for their antibacterial activity in the present investigation *H. pinifolia* and *H. stipulacea* were more superior to *C. serrulata* against the human pathogens in controlling their growth. The results of two ways ANOVA reveals that there is a significant differences ($P \leqslant 0.01$) in antimicrobial activity of all three extracts of all three seagrass extracts.

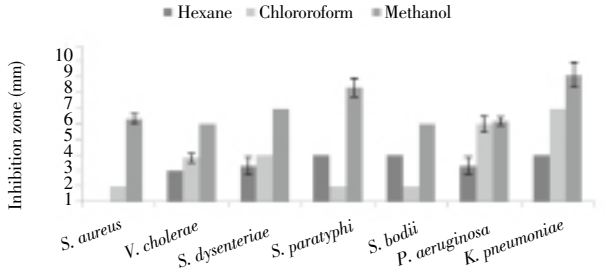

Figure 1. Antibacterial activity of crude extracts of *H. pinifolia* against human pathogens.

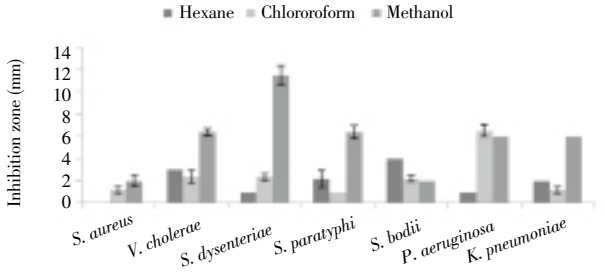

Figure 2. Antibacterial activity of crude extract of *H. stipulacea*

Table 1
Minimum inhibition concentration of seagrass extracts.

Seagrass extracts	Test pathogens	MIC(μg/mL)	Streptomycin(μg/mL)
Methanol extracts of *H. pinifolia*	*S. aureus*	150	120
Methanol extracts of *H. stipulacea*	*V. cholerae*	125	140
Methanol extracts of *H. stipulacea*	*S. dysentriae*	100	170
Methanol extracts of *H. pinifolia*	*S. paratyphi*	125	160
Chloroform extracts of *H. stipulacea*	*S. bodii*	100	140
Chloroform extracts of *C. serrulata*	*P.aeruginosa*	120	160
Methanolic extracts of *H. pinifolia* and chloroform extracts of *C. serrulata*	*K. pneumoniae*	100	140

against human pathogens.

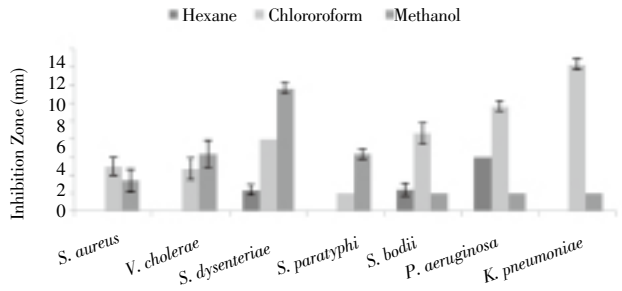

Figure 3. Antibacterial activity of crude extract of *C. serrulata* against human pathogens.

Among the three solvents tested methanol and chloroform extracts inhibited the growth of all the pathogens tested, of which methanol extracts are more active. Hexane extract of all the three seagrasses did not effective against *S. aureus*. Hexane extract of *C. serrulata* is also not active against *V. cholerae*, *S. paratyphi* and *K. pneumoniae*. The highest zone of inhibition (11 mm) was recorded in methanol extract of *H. stipulacea* against *S. dysenteriae* followed by (8 mm) with *H. pinifolia* and chloroform extract of *C. serrulata* against *K. pneumoniae*.

Minimum Inhibitory Concentrations of the methanol extracts of the three seagrasses which showed maximum activity against all pathogens tested (Table 1). The lowest MIC value (100 μg/mL) was in methanol extracts of *H. stipulacea* against *S. dysentriae*, and *K. pneumonia* and chloroform extract of *H. stipulacea* against *S. bodii*. Chloroform extract of *C. serrulata* inhibit the growth of *P. eruginosa* (120 μg/mL). Of the three seagrasses tested, minimum concentrations of *H. pinifolia* were effective in controlling the growth of *S. paratyphii* and *S. aureus*.

4. 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 seagrasses 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 seagrass extracts to produce bioactive compounds of potential therapeutic interest. Antimicrobial activities found in seagrasses were considered to be an indication of synthesis of bioactive secondary metabolites. The antibacterial activity of different extracts of *H. stipulacea*, *C. serrulata* and *H. pinifolia* on seven human pathogens are effective. Among them methanol was more effective than the others, this showed that methanol is suitable for extracting active compounds from seagrasses. The result of the present investigation is consistent to some earlier reports. Alam *et al*[6] 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*[10] reported *Halophila* and *Zostera* were more effective than *Cymodacea*. This also true in the present study, here *Halophila* and *Halodule* were active then *Cymodacea*. Premanathan *et al*[5] reported for the first time about the antiviral activity of seagrass *Thalossia hemprichii*, which is active against 4 pathogens among the 5 tested. Balasubramanian *et al*[15] screened 3 species of seagrasses against bacteria pathogens. The results indicated that the lipid and water-soluble phenolic extracts of both leaf and root-rhizome fractions of *H. pinifolia* are the most promising extracts as they showed a strong antibacterial activity against all the nine pathogens tested. This result is in agreement with the present finding that *H. pinifolia* showed maximum activity.

Hexane extracts of the seagrasses in the present investigation did not showed activity against some pathogens. As suggested by Schwarz and Noble[16] these bacterial strains may have some kind of resistance mechanisms *e.g.* enzymatic inactivation, target sites modification and decrease intracellular drug accumulation or the concentration of the compound used may not be sufficient. No inhibition was observed with negative controls, which proves that the solvents could not act as antibacterial agents. Besides, there are several factors such as the age of the plant, duration of storage, temperature, preparation of the media and pH which could indirectly affect the degree of antibiotic activity[17].

As for the effectiveness of the extraction methods, some

studies showed that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate[18] whereas in others, chloroform was better than methanol and benzene[19]. Kumar *et al*[10] recorded ethyl acetate and methanol extract of segrasses showed maximum activity against most of the pathogen and suggest ethyl acetate to be the best solvent for isolation of bioactive substance from seagrasses. Present study also in conformity with the earlier reports that, methanol extract of the test plants showed better antimicrobial activity than the other extracts. The variation in antibacterial activity of extracts might be due to distribution of antimicrobial substances, which varied from species to species as suggested by Lustigman and Brown[19]. The present investigation brings out adequate data on the antibacterial potential of seagrass extracts for the synthesis of novel antibiotics. Further research studies are being carried out on other species of seagrasses of different habitats in order to provide complete data of the antimicrobial potential of these plants. It is also essential to study the principle compound present in the seagrasses which is responsible for antimicrobial activity; it can be achieved by using advanced separation techniques.

Conflict of interest statement

We declare that we have no conflict of interest.

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