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Potential Antibacterial Activities of Seagrasses from Vellar Estuary; Southeast Coast of India

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Abstract: The present investigation carried out the antibacterial activity of seagrasses such as *Halophila ovalis* and *Halodule pinifolia.* Different organic solvents like diethyl ether, acetone and methanol were for the present study and tested the activity against *Acenetobacter* sp., *Salmonella typhii*, *Micrococcus* sp., *Shigella sonii*, *Vibrio cholerae*, *Staphylococccus* sp., *Proteus vulgaris*, *P. mirabilis*, *P. aeruginosa* and *Salmonella paratyphi*-B. The maximum antibacterial activity was recorded from the ethanol extracts of *H. pinifolia* against *Acinetobacter* sp., (20 mm), *Salmonella typhii* (20 mm), *Proteus mirabilis* (20 mm) and *Pseudomonas aeruginosa* (20 mm) showed the no activity against pathogens like *Vibrio cholerae* and *Salmonella paratyphi*-B.

Key words: Seagrasses · Different organic solvents · Vellar estuary and Antibacterial activity

INTRODUCTION

Seagrasses are marine angiosperms. They are sparsely distributed all over the sea except the polar regions. Seagrasses are not related to the grasses (Poaceae) family; which belonging to two families Viz., Hydrocharitaceae and Potamogetonaceae. Seagrasses are primary producers; they are highly productivity in origin. They supply as the basic energy source for more or less complicated food web.

Microbiologists and pharmacologists are having increased attention during the recent year towards seagrasses and marine algae, which constitute the potential bioactive substances. The last twenty five years there has been an exciting progress in the field of marine natural products. It has been realized that the many of these metabolites being biologically active are of biomedical importance and could be used as potential drugs. This subject is where marine ecology, the experimental chemistry, pharmacology and medicine use of algae in Indian ayurvedic system of medicine is little known.

Bioactive marine natural products play an important role in chemotherapy. The evidence for the use marine–flora marine algae to be precise in treatment of human ailments in extensive. In Asian maritime areas seagrass extracts were used as curative agents for various maladies such as antibiotics, antihelmintic, cough, antipyretic, antitumour, antidiarhoea, wound healing, treatment of gallstone and goiter etc.

The use of marine flora in the treatment of human ailments is extensive. There are only a very few reports concerning antifungal, antiviral, antibacterial activity of crude extracts of marine plants [1-4] including seagrasses and seaweeds. We can apply the familiarity of the antimicrobial activity for early selection of the seagrasses of active compounds [5]. Hence, an endeavor has been ended in the present study to investigate the antibacterial activity of seagrasses from Vellar estuary.

Description of the Study Area:

Vellar Estuary: Parangipettai is fortunately endowed with various aquatic biotopes, Viz., neritic, estuarine, backwater and mangrove, where the river vellar originating from the Servarayan hills of Salem district, covering a distance of 480 km meanders into the Bay of Bengal. A southern channel arising near the mouth of Vellar estuary leads to Killai backwaters and Pitchavaram mangroves, which in turn is connected to the Coleroon estuary that branches off from the river estuary.

The average depth of the estuary 2.5m and width 100 to 200 m. The sand bars appear at the mouth of the

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estuary. Their position and extent vary frequently due to tidal effect and water flow due to flooding during monsoon resulting in erosion and later accretion in summer. The large wetlands surrounding the estuarine complex are used for agricultural and aquaculture purposes. Drainage canals from aquaculture farms and domestic sewage are discarded in to the Vellar estuary.

MATERIALS AND METHODS

Collection of Samples: Live and healthy seagrass samples were collected during the low tide period. Then the samples were washed in seawater to remove epiphytes and extraneous matter. Then cleaned with freshwater to remove sand and salt so as to avoid pumping of the solvent during the extraction process. After draining off the water a known amount was taken for the preparation of extract and the rest was pressed with blotting paper and shade dried at room temperature and used for dry extract preparation.

Preparation of Extracts: Organic solvents like diethyl ether, acetone and methanol were used to prepare different extracts. All these solvents used were of analytical grade.

Dry Extraction: The present study following method of Padmini Sreenivasa Rao [6] was used for dry extraction. The dried seagrass samples were ground to coarse powder and packed in Soxhlet apparatus and extracted successively with diethyl ether, acetone and methanol for 36 to 48 hours at a room temperature of 50-55°C. The extracts were concentrated and dried under reduced pressure in a rotary evaporator and kept in deep freezer until tested.

Bacterial Strains Used: *Acenetobacter* sp., *Salmonella typhii*, *Micrococcus* sp., *Shigella sonii*, *Vibrio cholerae*, *Staphylococccus* sp., *Proteus vulgaris*, *P. mirabilis*, *P. aeruginosa* and *Salmonella paratyphi*-B. The microbial strains were obtained from the Department of Medical Microbiology (Rajah Muthiah Medical College and Hospital), Annamalai University, Annamalai Nagar, Tamil Nadu, India. The bacterial stock cultures were maintained on Mueller Hinton Agar medium at 4°C.

Antibacterial Assay: The bioassay was carried out using the agar disc diffusion method [7]. With paper disc of 6 mm diameter prepared from Whatman No: 1 filter papers. The antibacterial assay using gram$^+$ and gram$^-$ bacteria were carried out using the agar-plate method. The bacterial inocula was grown in nutrient broth overnight and a fixed volume inoculated into 10 ml aliquots nutrient agar, mixed and then poured over a nutrient agar base in sterile pretridishes; this formed the bacterial lawn. Initially both paper discs and well were used for testing the crude extracts. The paper disc of 6 mm diameter soaked in 6 µl of crude extract and placed on to the bacteria lawn after it had solidified, standard antibiotic disc used for control. The plates were incubated at 37°C overnight. The zone of inhibition was measured after 24 hours incubation.

RESULT

***Halophila ovalis*:** The extract obtained using ethanol showed a maximum activity against pathogens like *Acinetobacter* sp., (15 mm), *Salmonella typhii* (14 mm) and *Vibrio cholerae* (11 mm). Minimum activity against *Micrococcus* sp., (8 mm), *Shigella sonii* (7 mm), *Staphylococcus* sp., (7 mm), *Proteus vulgaris* (7 mm) and *Pseudomonas aeroginosa* (7 mm). Where it has no activity against *Proteus mirabilis* and *Salmonella paratyphi*-B.Observation made from methanol extract showed a maximum activity against *Salmonella paratyphi*-B (16 mm) and minimum activity against against *Acinetobacter* sp., (7 mm), *Micrococcus* sp., (7 mm), *Vibrio cholerae* (7 mm) and *Proteus mirabilis* (7 mm). It showed no activity against pathogens like *Salmonella typhii, Shigella sonii, Staphylococcus* sp., *Proteus vulgaris* and *Pseudomonas aeruginosa* (Table 1).

The extracts obtained using acetone showed a maximum activity against pathogen *Vibrio cholerae* (14 mm) showing a minimum activity against pathogen like *Acinetobacte*r sp., (8 mm), *Salmonella typhii* (7 mm), *Micrococcus* sp., (7 mm) and *Pseudomonas aeruginosa* (8 mm) showed no activity against pathogen like *Shigella sonii, Staphylococcus* sp., *Proteus vulgaris, Proteus mirabilis* and *Salmonella paratyphi*-B.

Dichloroethane showed a minimum activity against pathogens like *Vibrio cholerae* (7 mm), *Staphylococcus* sp., (7 mm), *Proteus mirabilis* (5 mm), where as showed no activity pathogens like *Micrococcus* sp., *Shigella sonii, Pseudomonas aeruginosa, Acinetobacte*r sp., *Proteus vulgaris, Salmonella typhii, Salmonella paratyphi*-B.

***Halodule pinifolia*:** The investigation made on ethanol extracts records maximum activity against *Acinetobacte*r sp., (20 mm), *Salmonella typhii* (20 mm), *Micrococcus* sp., (18 mm), *Shigella sonii* (15 mm), *Staphylococcus* sp., (18 mm), *Proteus vulgaris* (16 mm), *Proteus mirabilis*

Table 1: Shows the antibacterial activity of *Halophilla ovalis* against human pathogens

S. No	Pathogens	Solvent used (Zone of inhibition mm in diameter)			
		Acetone	Methanol	Ethanol	Dichloroethane
1.	*Acinetobacter* sp	8	7	15	-
2.	*Salmonella typhii*	7	7	14	-
3.	*Micrococcus* sp	7	-	8	-
4.	*Shigella sonii*	-	7	7	-
5.	*Vibrio cholerae*	14	-	11	7
6.	*Staphylococcus* sp	-	-	7	7
7.	*Proteus vulgaris*	-	-	7	-
8.	*Proteus mirabilis*	-	7	-	5
9.	*Pseudomonas aeruginosa*	8	-	7	-
10.	*Samonella paratyphi*-B	-	16	-	-

Table 2: Shows the antibacterial activity of *Halodule pinifolia* against human pathogens

S. No	Pathogens	Solvent used (Zone of inhibition mm in diameter)			
		Acetone	Methanol	Ethanol	Dichloroethane
1.	*Acinetobacter* sp	-	12	20	-
2.	*Salmonella typhii*	15	-	20	14
3.	*Micrococcus* sp	13	15	18	14
4.	*Shigella sonii*	14	12	15	13
5.	*Vibrio cholerae*	-	-	-	-
6.	*Staphylococcus* sp	17	-	18	13
7.	*Proteus vulgaris*	14	10	16	12
8.	*Proteus mirabilis*	14	14	20	12
9.	*Pseudomonas aeruginosa*	13	18	16	15
10.	*Samonella paratyphi*-B	-	-	-	-

(20 mm), and *Pseudomonas aeruginosa* (20 mm) showed the no activity against pathogens like *Vibrio cholerae* and *Salmonella paratyphi*-B.

The extracts obtained showed a maximum activity against pathogen like *Acinetobacte*r sp., (12 mm), *Micrococcus* sp., (15 mm), *Shigella sonii* (12 mm), *Staphylococcus* sp., (12 mm), *Proteus vulgaris* (10 mm), *Proteus mirabilis* (14 mm) and *Pseudomonas aeruginosa* (12 mm) showed the no activity against pathogens like *Vibrio cholerae* and *Salmonella paratyphi*-B (Table 2).

Acetone extract obtained maximum activity against *Salmonella typhi* (15 mm), *Micrococcus* sp. (13 mm), *Shigella sonii* (14 mm), *Staphylococcus* sp., (17 mm), *Proteus vulgaris* (14 mm), *Proteus mirabilis* (14 mm) and *Pseudomonas aeruginosa* (18 mm) showed no activity against pathogens like *Acinetobacte*r sp., *Vibrio cholerae* and *Salmonella paratyphi*-B.

The extract obtained from dichloroethane showed a maximum activity against pathogens like *Shigella sonii* (13 mm), *Staphylococcus* sp., (13 mm), *Proteus vulgaris* (12 mm), *Proteus mirabilis* (12 mm) and *Pseudomonas aeruginosa* (15 mm), *Salmonella typhi* (14 mm), *Micrococcus* sp., (14 mm), whereas showed no activity against pathogens like *Acinetobacte*r sp., *Vibrio cholerae* and *Salmonella paratyphi*-B.

DISCUSSION

The degree of antibiotic property depends upon the suitable solvents used for extraction; it could also depend upon the condition or state of the sample along with the season in which alga was collected. There are several factors such as age of the plant duration of storage, temperature, preparation of the media and pH, which sometimes indirectly [8]. Martinez-Nadal *et al.,* [9] mentioned that benzene and diethyl ether were suitable solvents for extracting on antibiotic principle [10] used acetone for extraction of an active principle. Parekh [11] reported that seaweed extracts prepared with chloroform showed less activity. Our present investigation different

from earlier investigation; here ethanol and methanol extraction showed better zone of inhibition against bacterial pathogens.

Hornsey and Hide [12] used acetone as a solvent for extracting antimicrobial compounds from British marine algae. Shelat [13] found methanol and diethyl methyl formide extracts of seagrass sp., were active against gram$^+$ bacteria. In the present investigation the ethanol, methanol, acetone and dichloroethane extract of algae used for screening experiment. The results shown that seaweeds have very active against several gram$^+$ and gram$^-$ bacteria.

Selvi *et al*., [14] screened around 20 algae using methanol and ethanol along Idinthakarai coast and they reported that *Bacillus subtilis* and *Staphylococcus* sp., were highly susceptible to most of the algal extracts. In the present investigation the ethanol extract showed less activity against *Staphylococcus* sp.

In the present study of seagrass *Halodule pinifolia* shows promising, results against antibacterial pathogens. This finding lends support to that of [13] who demonstrated that some marine plants showed antibacterial activity against three bacterial strains.

Prabhadevi *et al.,* [4] recorded negative antibacterial activity in *Halophila ovalis* while this slightly similar to the previous study where only ethanol extrtacts showed minimum activity while methanol, acetone and dichloroethane showed trace activity.

CONCLUSION

The commercial antibiotics are highly effective to kill the bacterial pathogens involved in common infection. The solvent extracts of two different seagrasses used in the present study showed significant bacterial action. The interest information is that the product in the form natural good for health and fails to cause side effects. From these preliminary investigations the algal members of both the estuaries merit further investigation. Further, a detailed study to correlate the physiological stages of the algae. With their antibacterial activity is essential.

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