Research Paper

Volume : 4 | Issue : 5 | May 2015 • ISSN No 2277 - 8179

Antimicrobial Drug Possibility from Marine Macroalgae & selected Flora from Sunderban Delta

Healthcare

KEYWORDS : Mangroves, Sunderbans, Antimicrobial activity, Seaweeds

Madhumita Mukherjee Executive Director, NFDB

Reshma Sarkar Aquatic Resource Health Management Center Department of Fisheries, Govt. of West Bengal Pailan

ABSTRACT

Marine organisms are a rich source of structurally novel and biologically active metabolites. As a result of evolving resistance of microorganisms to existing antibiotics, there is an increasing need for newer antibiotics, the present study was carried out to investigate the antimicrobial potentiality of the marine algae Enteromorpha intestinalis ,Catenella impudica, Ulva sp.against strains of Gram positive, Gram negative bacteria such as Vibrio chlolerae ,Enterococcus faecalis ,Shigella boydii, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus organisms that cause diseases and disorders in man, animals and plants. As for the mangroves of Indian Sunderbans such as Acanthus illicifolius, Heritiera fomes, Aegiceras corniculatum, Xylocarpus granatum, Sonneretia apetala, Ceriops decandra, test organisms include, Bacillus subtilis, Staphylococcus aureus,Streptococcus pneumoniae, Enterococcus faecalis and Micrococcus luteus among the gram positive bacteria and from gram negative bacteria Proteus mirabilis ,Salmonella typhi, Shigella flexineri, Vibrio cholerae, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa ,Aeromonas hydrophilia. Crude extracts revealed a wide range of antimicrobial activity against tested pathogens .The overall antimicrobial activity assessed from the above results indicates the presence of active constituents in the extractions of seaweeds which can be exploited for the production of lead molecules which are of use in pharmaceutical industry

Introduction

Marine environment is an exceptional reservoir of biologically active natural product, many of which exhibit structural features not been found in terrestrial natural products. Marine algae or Seaweed, the most accessible marine resources of the coastal zone occupy potentially important place as a source of biomedical compounds1. They are the only source for the production of Phytochemical such us agar, carrageenan and algin also contain many trace elements, minerals, protein, iodine, bromine, vitamins and many bioactive substances. A marine alga was first investigated for the antibiotic activity in 1951 and since then; many works have been carried out on the antibacterial activity of marine plants. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. To date, many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used 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. In the present study, we describe the anti-microbial characteristics of methanol, and ethanol extracts of some marine algae obtained from the Indian Sunderbans. Mangrove plants have been used in folklore medicines and extracts from mangrove species have proven inhibitory activity against human, animal and plant pathogens. Several species of mangrove produce bioactive compounds that may control microbial growth. Antimicrobial activities of plant constituents such as phenol, quinines, flavones, flavor-noids, tannins, terpenoids, essential oils and alkaloids have been reported by several authors (Weimann andHeinrich, 1997; Atindehou et al., 2002; Edeoga et al., 2005). There is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and reemerging infectious diseases (Rojas et al., 2003). The present study made an attempt to find out the antibacterial activity of 7 mangrove plants.

Materials and Methods:

Study site: The Indian Sundarbans (within the latitude 21°13_N to 22°40_N and longitude 88°03_E to 89°07_E) at the apex of the Gangetic delta is one of the most biologically productive and taxonomically diversified, low line, mangrove detritus

based, open, dynamic, heterogeneous coastal ecotone. This mangrove forest has been declared as the World Heritage Site by IUCN in 1987, Biosphere Reserve under Man and Biosphere Programme by UNESCO in 1989 and is a proposed RAMSAR site. The climate of the area is humid (upto 96%), tropical with temperature ranging from 11.8°C to 34.5°C. The climate is monsoonal with an average rainfall of 1900mm during monsoon months (July–October). The samples both algae and flora were collected during the months of February-june 2011.

Plant materials:

Algal samples used were *Enteromorpha intestinalis ,Catenella impudica, Ulva* sp.

Mangroves used were namely *Acanthus illicifolius, Heritiera fomes, Aegiceras corniculatum, Xylocarpus granatum, Sonneretia apetala, Ceriops decandra Bruguiera gymnorrhiza* .

Microorganism used for the activity test:

Both gram positive and gram negative bacterial strains were taken for the test. The bacterial strains used for the investigation are *Bacillus subtilis, Staphylococcus aureus,Streptococcus pneumoniae, Enterococcus faecalis and Micrococcus luteus* among the gram positive bacteria and from gram negative bacteria *Proteus mirabilis ,Salmonella typhi, Shigella flexineri, Vibrio cholerae, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa ,Aeromonas hydrophilia Vibrio parahaemolyticus,*. These organisms were isolated from diseased aquatic animals and water which are maintained at ARHMC, Pailan. The bacterial stock cultures were maintained at 4° C.

Preparation of Plant Extracts:

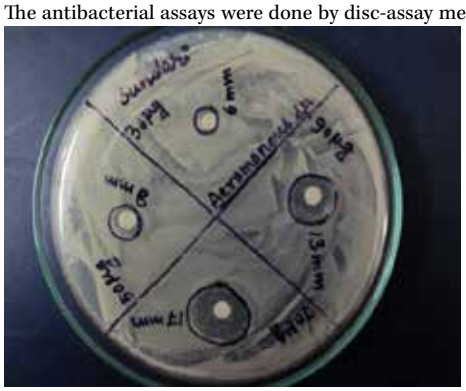
The plant material were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight plastic container. About 200 gm of plant powder are taken for extraction with either ethanol or methanol(listed in table I) for 3-4 times at room temperature. The extracts were combined and evaporated to dryness under reduced pressure

(temperature kept below 50° C) The crude extracts thus obtained were used in the primary antibacterial assay test as this method of drying was confirmed to be most suitable for testing against bacteria. The above method was advantageous as minimum degradation occurred during shade drying (padmini Sreenivasa rao *et al.*).

Plant /part used	Solvent used for extraction
Enteromorpha intestinalis thallus	Methanol
2. *Catenella impudica* (thallus)	Methanol
3. *Ulva* (thallus)	Methanol
4. *Acanthus illicifolius* (leaves)	Methanol
5. *Heritiera fomes* (leaves)	Methanol
6. *Aegiceras corniculatum* (leaves)	Ethanol
7. *Xylocarpus granatum* (fruit inner layer)	Ethanol
8. *Xylocarpus granatum* (fruit outer layer)	Ethanol
9. *Xylocarpus granatum* (seed)	Ethanol
10. *Sonneretia apetala* (fruit)	Ethanol
11. *Sonneretia apetala* (leaves)	Ethanol
12. *Ceriops decandra* (leaves)	Ethanol
13. *Bruguiera gymnorrhiza*(leaves)	Ethanol

Antibacterial Assay:

The antibacterial assays were done by disc-assay method (Casida



1986). In short, 100 mg of each extract dissolved in appropriate solvent 10ml ie,stock solution of 1000µg/ml was prepared from which serial dilutions of 800µg/ml,600µg/ml,400µg/ml,200 µg/ml were prepared applied to sterile filter paper discs (6 mm). The antibacterial assay using gram +ve and gram &ve bacteria, were carried out using the agar plate method. After allowing the solvent to evaporate, the discs were placed on nutrient agar test plates (Himedia, India). A disc loaded with only solvent was similarly prepared as a control. The plates were incubated overnight at 37°C. The zone of inhibition of bacteria around the disc was measured in millimetres, a clear zone around a disc was evidence of antimicrobial activity. Each test was prepared in duplicate. Discs loaded with the extracting agents were tested as controls.

The picture shows an agar plate indicating the Inhibition zones due to the antibacterial effect of ***Heritiera fomes*** (local name -Sundari) against ***Aeromonas hydrophilia***

Result:

Table I Showing Inhibition Zones Due To Antibacterial Effect Of The Algal And Floral Samples

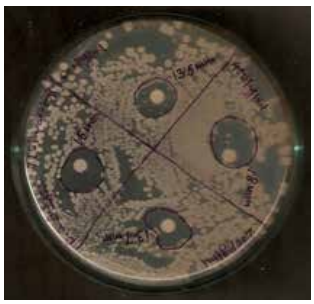
Sl. No.	Date of Inoculation	Extract used	Bacterial Strain used	Dose (u g / ml)	Sensitive zone (mm)
1.	7.6.11	Bruguiera gymnorrhiza	Aeromonous hydrophilia	200 400 600 800	17.5 17 17.5 13
2.	8.6.11	*Xylocarpus granatum* (seed)	Aeromonous hydrophilia	200 400 600 800	15 18 20 24
3.	8.6.11	Xylocarpus granatum (outer layer)	Aeromonous hydrophilia	200 400 600 800	13.7 18 13.5 15
4.	8.6.11	*Xylocarpus granatum* (inner layer)	Aeromonous hydrophilia	200 400 600 800	14 12.5 22 14
5.	23.6.11	Catenella impudica	Aeromonous hydrophilia	200 400 600 800	9 9 7 7
6.	23.6.11	Enteromorpha intestinalis	Aeromonous hydrophilia	200 400 600 800	8 9 14 10
7.	24.6.11	*Xylocarpus granatum* (seed)	Vibrio parahaemolyticus	200 400 600 800	2.5 3.5 2.5 ----
8.	24.6.11	Xylocarpus granatum (outer layer)	Vibrio parahaemolyticus	200 400 600 800	4 3.5 2.5 2
9.	24.6.11	Xylocarpus granatum (inner layer)	*Vibrio parahaemolyticus*	200 400 600 800	---- ---- 2 ----
10.	16.8.11	Enteromorpha intestinalis	Enterococcus faecalis	200 400 600 800	11 8 11 18
11.	16.8.11	Enteromorpha intestinalis	Escherichia.coli	200 400 600 800	11 11 11.5 13
12.	16.8.11	Ulva clathrata	Escherichia.coli	200 400 600 800	7 6.6 8 10.5
13.	16.8.11	Heritiera fomes	Aeromonous hydrophilia	200 400 600 800	6 8 17 13

Plates showing invitro activity :

Effect of Enteromorpha intestinalis against Enterococcus faecalis

Effect of Catenella impudica against Aeromonous hydrophilia

Volume : 4 | Issue : 5 | May 2015 • ISSN No 2277 - 8179

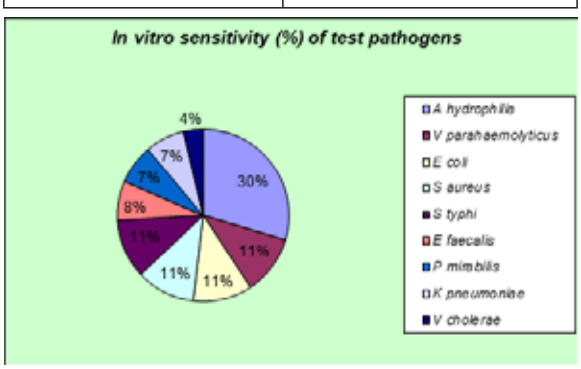


Effect of Xylocarpus granatum(outer layer) against Aeromonous hydrophilia

Table II : Showing in-vitro antibacterial activity of certain mangrove flora .

N.B: all processes have been done at the laboratory of ARHMC ,Pailan,. The in-vitro activity have been confirmed from the National Institute of Ocean Technology, Chennai.

Floral sample	Activity against
Acanthus illicifolius (leaves)	Staphylococcus aureus
Ceriops decandra (leaves)	Salmonella typhi
Sonneretia apetala (leaves)	Proteus mirabilis
Sonneretia apetala (fruit)	Enterococcus faecalis Klebsiella pneumoniae Micrococcus luteus Proteus mirabilis Salmonella typhi Staphylococcus aureus Vibrio cholerae
Aegiceras corniculatum(leaves)	Aeromonous hydrophilia Escherichia coli Staphylococcus aureus Salmonella typhi Klebsiella pneumoniae



Discussion:

The antimicrobial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plants, experimental methods, etc. The detailed results of the inhibition zones formed due to antibacterial activity of the selected samples are shown in Table I and II. Among the seaweeds *Ulva clathrata* was effective against *Escherichia.coli and* was most effective at 600 (ug/ml) concentration. *Enteromorpha intestinalis* showed antibacterial activity against *Enterococcus faecalis(*most effective at 800 ug/ml*), Escherichia.coli (*most effective at 600 ug/ml*), as well as Aeromonous hydrophilia(*most effective at 600 ug/ml*). Catenella impudica* was active against *Aeromonous hydrophilia* at a concentration of 200 ug/ml.

The mangroves *Aegiceras corniculatum* and *Sonneretia apetala* showed broad-spectrum antibacterial activity against both gram positive and gram-negative bacteria. Though the leaf extracts of *Sonneretia apetala* were less effective than the fruit extract. The results are shown in table II. All of the three floral parts of *Xylocarpus granatum,* i.e, fruit inner layer, fruit outer layer as well as seed were effective against *Aeromonous hydrophilia* and *Vibrio parahaemolyticus* though the activity was stronger against Aeromonous *hydrophilia* for all the three parts. *Bruguiera gymnorrhiza* showed activity against *Aeromonous hydrophilia* most effective at 200 200 ug/ml concentration. *Heritiera fomes* showed activity against *Aeromonous hydrophilia* most effective at 600 ug/ml concentration .So from the above data it can be concluded that most of the mangroves are effective against the gram negative bacterium *Aeromonous hydrophilia* i.e ,it is the most susceptible organism among the selected pathogens . In our study, some of the bacterial strains did not respond to crude extracts. This might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract or synergism by the presence of some compounds or factors in the extract. The variation of antibacterial activity of our extracts might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Similar observations were made by Vlachos *et al.* (1997) . Our results also showed that the sensitivity of pathogens is more to mangrove extracts compared to algal extracts.

From the above preliminary studies on the antibacterial activities, all the crude extractions of seaweeds showed promising activity against a number of the test pathogens, promising a future scope for the use of marine seaweeds against a wide range of microbial populations. The work can further be extended to reveal its vast sources of secondary metabolites that attributes to the antimicrobial activity. The result of the antimicrobial activity expressed in term of diameter of zone of inhibition in millimeter. The economical uses of products from mangrove ecosystems are many and varied. Traditionally, the mangroves have been exploited for firewood and charcoal. Use has also been found for mangroves in the construction of dwellings, furniture, boats and fishing gear, tannins for dyeing and leather production. The mangroves provide food and wide variety of traditional products and artifacts for the mangrove dwellers. Extracts and chemicals from mangroves are used mainly in folkloric medicine (e.g. bush medicine), as insecticides and pesticides and these practices continue to this day. Overall, the present study provides enough data to show the potential of mangrove and algae extracts for development of anti-pathogenic agents for a variety of uses. Ecologically, there is likelihood that the secondary metabolites / PUFAs produced by the different marine flora may have a role in fish growth and protection from diseases. The renewable / cultivable nature of marine flora is another advantage for development of potential antibacterial products for use in feed or by other means administration in aquaculture. Economically feasible standard operating procedures can be developed in preparing the extracts/fractions in large scale with reproducible antibacterial efficiency. However the extraction of novel natural chemical compounds from mangroves, in addition to those already known to the pharmacopoeia of the people is in its infancy. A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of new therapeutic agents, but because such information may be of value in disclosing new sources of already known biologically active compounds. Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.

Acknowledgement:

The authors would like to thank

Ministry of Earth Science ,Govt. of India, Under the project *Survey & Isolation of bioactive compounds from selected flora & fauna in Sunderban Biosphere*

Dr.R Kirubagaran, of National Institute of Ocean Technology ,Chennai for confirming the invitro tests.

Dr. A.K Siddhant of CSMCRI,Central Salt and Marine Chemicals Research Institute, Bhavnagar for the identification of algal samples.

REFERENCE

1. Aarati S. Yeragi, Rajendra Nayak and S.G. Yeragi, 2002. Composition and present status of mangroves at Achara creek. In: Goldin Quadros (ed.), Proceedings of the National Seminar on Creeks, Estuaries and Mangroves, Pollution and Conservation. Zoology Department, Thane, 240-242 | 2. Abhijit Mitra and D.P. Bhattacharryya, 2001. Biological diversity of mangrove wetlands in Sundarbans and scope for alternative source of income. Everyman's Science, 36(2): 71-75 | 3. A F M Shahid-Ud-Daula And Mohammad Anwarul Basher Phytochemical Screening, Plant Growth Inhibition, And Antimicrobial Activity Studies Of Xylocarpus Granatum; Malaysian Journal Pharmaceutical Sciences, Vo. 7, No. 1, 9–21 (2009) | 4. Alongi, D.M., G. Wattayakorn, T. Ayukai, B.F. Clough, E. Wolanski and G.J. Brunskill, 2000. An Organic carbon budget for Mangrove - Fringed Sawi Bay, Southern Thailand. Phuket marine biological Center Special Publication, 22: 79 - 85. | 5. Atakan SUKATAR, N. Ulku KARABAY-YAVAS, OGLU*, Guven OZDEMIR, Zerrin HORZUM Antimicrobial activity of volatile component and various extracts of Enteromorpha linza (Linnaeus) J. Agardh from the coast of Izmir, Turkey; Annals of Microbiology, 56 (3) 275-279 (2006) | 6. Bandaranayake, W. M, 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management, 10: 421 - 452. | 7. Blasco, F., and M. Aizpuru, 2002. Mangroves along the coastal stretch of the Bay of Bengal: Present status. Indian Journal of Marine Sciences, 31(1): 9 - 20. | 8. Borkar, M.U., R.P. Athale and Goldin Quadros, 2006. Occurrence of heavy metals in abiotic and biotic components of the Mangrove ecosystem of Thane creek. Ecology, Environment and Conservation, 12(4): 723-728. | 9. Chandrasekaran, M., V. Venkatesalu, M. Anantharaj, S. Rajendran and K. Prabhakar, 2004. Studies on the antibacterial activity of a mangrove, Aegiceras corniculatum . Journal of the Annamalai University, Science, pp. 169 – 174 | 10. Chandrasekaran, M., V. Venkatesalu, S. Sivasankari, K. Kannathasan, A.K. Sajitkhan, K. Prabhakar, S. Rajendran and Y. Lakshmi Sarayu, 2006. Antibacterial activity of certain mangroves against methicillin resistant Staphylococcus aureus . Seaweed Res. Utiln., 28(1): 165-170. | 11. Ghoshal Chaudhuri, S., R. Dinesh, R. Raja, Shashi Kumar and N. Ravisankar, 2005. Physico-chemical, biochemical and microbial characteristics of soils of mangroves of South Andaman: Impacts of Anthropogenic disturbances. The Indian Forester, 131(5): 660 - 666. | 12. G. Karthikaidevi, K. Manivannan, G.Thirumaran, P. Anantharaman and T. Balasubaramanian Antibacterial Properties of Selected Green Seaweeds from Vedalai Coastal Waters; Gulf of Mannar Marine Biosphere Reserve; Global Journal of Pharmacology, 3 (2): 107-112, 2009 | 13. G.Subba Rangaiaha, P. Lakshmi* a , K. Sruthikeerth Antimicrobial activity of the crude extracts of Chlorophycean seaweeds Ulva, Caulerpa and Spongomorpha sps. against clinical and phytopathogens; Drug Invention Today 2010, 2(6),311-314 | 14. Hanaa H. Abd El-Baky, Farouk K. El Baz and 2Gamal S.E l Baroty Evaluation of Marine Alga Ulva lactuca L. as A Source of Natural Preservative Ingredient; American-Eurasian J. Agric. & Environ. Sci., 3 (3): 434-444, 2008 | 15. Jiny Varghese K1*, Belzik N1, Nisha A R1,Resiya S 1, Resmi S 1,Silvipriya KS Pharmacognostical and phytochemical studies of a Mangrove (Sonneratia caseolaris) from Kochi of Kerala state in India; Journal of Pharmacy Research 2010, 3(11),2625-2627 | 16. Joseph Selvin* and Aaron Premnath Lipton Biopotentials Of Ulva Fasciata And Hypnea Musciformis Collected From The Peninsular Coast Of India; Journal of Marine Science and Technology, Vol. 12, No. 1, pp. 1-6 (2004) | 17. M. A. Alam, M. Sarder, M. A. Awal, M. M. H. Sikder And K. A. Daulla Antibacterial Activity Of The Crude Ethanolic Extract Of Xylocarpus Granatum Stem Barks; Bangl. J. Vet. Med. (2006). 4 (1): 69–72 | 18. Natarajan Arivuselvan, Durai Silambarasan, Thangavel Govindan and Kanthasamy Kathiresan Center of Advanced Study in Marine Biology | 19. Antibacterial Activity of Mangrove Leaf and Bark Extracts Against Human Pathogens; Advances in Biological Research 5 (5): 251-254, 2011 Pradeep V. Khajure and J.L. Rathod Department of Marine Biology, Karnatak University P.G and Research Centre, Karwar 581303, Karnataka | 20. Recent Research in Science and Technology 2010, 2(6): 98-99 Antimicrobial Activity Of Extracts Of Acanthus Ilicifolius Extracted From The Mangroves Of Karwar Coast Karnataka. | 21. Shaikh J. Uddin,1 I. Darren Grice,2 and Evelin Tiralongo Cytotoxic Effects of BangladeshiMedicinal Plant Extracts, School of Pharmacy, Griffith University, Gold Coast campus, 4222,Queensland, Australia Institute for Glycomics, Griffith University, Gold Coast campus, Australia, 21 July 2009 | 22. S. Ganesh,* and J. Jannet Vennila: Screening for Antimicrobial Activity in Acanthus ilicifolius Department of Bioinformatics, Karunya University, Coimbatore, Tamilnadu, India 2010, 2 (5):311-315 | 23. S. Choudhury, A. Sree1, S.C. Mukherjee, P. Pattnaik And M. Bapuji, Asian Fisheries Science 18 (2005): 285-294 Asian Fisheries Society, Manila, Philippines In Vitro Antibacterial Activity of Extracts of Selected Marine Algae and Mangroves against Fish Pathogens | 24. S. Ravikumar1*, M. Gnanadesigan1, P. Suganthi1 and A. Ramalakshmi International Journal of Medicine and Medical Sciences Vol. 2(3) pp. 94-99, March 2010 Antibacterial Potential Of Chosen Mangrove Plants Against Isolated Urinary Tract Infectious Bacterial Pathogens | 25. Varahalarao Vadlapudi and K Chandrasekhar Naidu In vitro antimicrobial activity Ceriops Decandra against Selected Aquatic, Human and Phytopathogens; International Journal of ChemTech Research CODEN (USA): IJCRGG ISSN : 0974-4290 Vol.1, No.4, pp 1236-1238, Oct-Dec 2009 |