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Research Article

Antibacterial Activity of the Marine Diatom *Skeletonema costatum* Against Selected Human Pathogens

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ABSTRACT

In recent years, biological activities, potential health benefits, and nutritional value of marine algae have been intensively investigated. Marine algae represent about 9% of biomedical compounds obtained from the sea. Infectious diseases caused by bacteria have a large impact on public health. The resistance of pathogenic bacteria to existing antibiotics has become a global epidemic. Marine algae derivatives have shown promise as candidates in novel, antibacterial drug discovery. The antibacterial activity of the marine diatom *Skeletonema costatum* (Greville) Cleve collected from South East Coast of India was examined against the selected Human Pathogenic bacteria. The active compounds extracted using water, ethanol and methanol partly purified from the algal extract were tested against the pathogens: *Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Salomonella typhi, Salmonella paratyphi B, Vibrio cholerae and Staphylococcus aureus. T*he organic extract B (Methonol) appeared to be the most active. The extract B exhibited weak activity against *Proteus mirabilis, Proteus vulgaris,Pseudomonas aeroginosa, Salmonella paratyphi B and Vibrio cholerae*. Extract B exhibited it maximum activity against *Staphylococcus aureus.* It exhibited no activity against *Salmonella typhi*, the fraction C (Ethanol) appeared to inhibit *Proteus mirabilis, Samolnella typhi* and *Staphylococcus aureues* weakly, whereas *Vibrio cholerae* was inhibited effectively by this fraction. The growth of the Human pathogenic bacteria appeared to be inhibited by the organic extract of the diatom. However, there was no activity in distilled water extracts against all pathogens tested. Hence from the observed results The Extract B extract was found to be potent against mentioned human pathogens. They could be used by the pharmaceutical industry in drug development to treat diseases like cancer, acquired immune-deficiency syndrome (AIDS), infection from virus, bacteria and fungus, inflammation, pain, arthritis etc.

Keywords: Diatom, algal extract, bacteria, human pathogen, *Skeletonema costatum*

INTRODUCTION

Natural products have been known as premier sources that can produce biologically active secondary metabolites which are potential chemotherapeutic agents. Mankind has observed for about thousands of years that marine fauna and flora contain substances with potent antimicrobial activity. Since then, all the life forms from marine environment have been thoroughly investigated for the content of such natural products. Marine organisms are rich sources of structurally novel and biologically active metabolites. So far, many chemically unique compounds of marine origin with different biological activity have been isolated and a number of them are under investigation and/or being developed as a new pharmaceutical product. Discovering new therapeutic molecules is becoming increasingly important as more and more bacteria become resistant to the usual antibiotics. Traditionally used in Asiatic medicines, algae, since the second half of the 20th century, are screened for their biological activities. Thus, antibacterial effects have been noticed in all the algal classes and notably in diatoms, the major component of the phytoplankton (Burkholder et al., 1960; Aubert and Gauthier 1966; Duff et al., 1966; Aubert et al., 1968a, b; Aubert and Gambarotta 1972; Berland et al., 1972; Aubert et al., 1979; Gauthier, 1980; Cooper et al., 1983; Pesando 1990). Although extremely effective, antibiotics are able to induce resistance in bacteria. For 450 years, bacterial resistance has been the main factor responsible for the increase of morbidity, mortality and health care costs of bacterial infections. The defense mechanism against antibiotics is widely present in bacteria (e.g. Pseudomonas, Klebsiella, Enterobacter, Acinetobacter, Salmonella, Staphylococcus, Enterococcus and Streptococcus) and became a world health problem (Clementino, 2005). Several algal species contain natural bioactive compounds that act as potent antimicrobial agents (Ozdemir 2004; Khan 2006). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. In recent years, use of sea plants (macro algae, sponges, micro algae) as an effective alternative to antibiotics has gained importance specially to combat disease problem. Bacterial infection causes high rate of mortality in human population and aquatic organisms. Development of multidrug resistant bacteria due to uncontrolled usage of chemotherapeutic agents is a serious issue posing danger to aquatic animals and human

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Table 1: shows the result of the *in vitro* testing of extracts against pathogenic bacteria.

Organism	Extract A (Water)	Extract B (Methanol)	Extract C (Ethanol)
Proteus mirabilis	-	+	+
Proteus vulgaris	-	+	-
Pseudomonas aeuroginosa	-	+	-
Salmonella typhi	-	-	+
Salmonella paratyphi B	-	+	-
Staphylococcus aureus	-	++	+
Vibrio cholerae	-	+	++

(-): No Activity
(+): 0<D<9mm
(++): 9< D<15 mm
(+++): D >15mm
D: Diameter of the inhibition zone in millimeters including the diameter of the disc.
The marine diatom collected from South Coast of India, has shown to possess the antibacterial activity, (against *Proteus mirabilis, Proteus vulgaris,Pseudomonas aeuroginosa, Salmonella paratyphi B,Staphylococcus aureus,Vibrio cholerae the highest being Staphylococcus aureus.*

health. Prokaryotic and eukaryotic microalgae produce a wide array of compounds with biological activities. These include antibiotics, algicides, toxins, pharmaceutically active compounds and plant growth regulators. Toxic microalgae, in this sense, are common only among the Cyanobacteria and Dinoflagellates. The microalgal toxins are one of the greatest mysteries in the world of bio toxicology deserving significance as material for developing useful drugs.

Microbial diversity encompasses the ecosystems and ecological processes of which they are a part. Marine environment can be considered as Ocean of diversity. There is indeed a genetic, physiology and biochemical diversity superior to that observed on the continents. These characteristics of the marine environment contribute an immense filed of investigation. Phytoplankton form the base of the ocean food web and they are responsible for approximately one -half of aquatic photosynthetic and play a key role in stabilizing atmospheric carbon dioxide. These organisms show an immense diversity to form, pigmentation and cellular structure, which are all adaptation to living in the oceanic environment. There are several relationships between phytoplankton and bacteria in aquatic ecosystems which may range from symbiosis to parasitism. Marine algae collected from Indian coast have been shown to possess a number of antimicrobial activities. Discovering therapeutic molecule is becoming increasingly important as more and more bacteria become resistant to the usual antibiotics. Antibacterial effects have been noticed in all the algal classes and notably in diatoms. Antibacterial study is desirable not only to contribute towards an understanding of ecological interactions but also to assess the potentials of algal antimicrobial activity and their possible therapeutic value (McN Sieburth, 1964). The aim of this study is to investigate the efficacy of different extracts of *Skeletonema costatum* against human pathogens.

MATERIALS AND METHODS

All solvents used are of analytical grade. Chemicals and Media used were purchased from Sigma and Himedia.

Sampling and Identification.

Sampling was made for collection of data on phytoplankton for qualitative and quantitative analysis. Phytoplankton was collected from the surface water using no.30 phytoplankton net made from the molting silk (mesh size 68 μm). Diatoms were collected from the surface water using no.10 phytoplankton (mesh size 58μm). Identification of the diatom was based on the present taxonomic identification of various groups, genus, and species of phytoplankton occurring in the marine environment of East Coast of India mainly based on their external features. (Dr. Govindasamy, Research Associate, University of Madras). The diatom *Skeletonema costatum* was grown axenically in sterile F/2 medium (Guillard and Ryther,1962) using the reconstituted sea water component. Cultures were maintained unshaken in an incubator at 24° C with illumination from cool white fluorescent bulbs on a 12:12 light/dark cycle. This light level was used for an extended period and the diatom grew well under this condition, although the doubling time of the cultures was probably not optimal. Approximately every 8 days, cells were transferred to 250 ml foam stoppered Erlenmeyer flasks containing 100 ml fresh sterile F/2 medium to key cells in exponential growth phase (Jeffery M. Rouse et al 2002).

Preparation of Algal Extract.

The diatom in exponential growth phase was recovered from culture by batch centrifugation at low speed. The resultant algal pellet was then immediately lyophilized before extraction. Algal powder was re-suspended by stirring in ethanol 95% with an ultra -turrax 930 min), The alcohol extract was centrifuged to remove cellular materials. They were combined and evaporated under vacuum at low temperatures. Distilled water was then added and partitioned with methylene chloride. The organic phases were collected and concentrated under vacuum at low temperatures.

Purification

The organic extract A was solubilized in methylene chloride (150 ml/g) and partitioned by NaOH (0.5 N,6 x

60ml/g). The aqueous phases were combined, neutralized by HCl (12 N) and partitioned again with methylene chloride (6x 55 ml/g). The organic phases resulting from the neutralization were collected and concentrated under vacuum at low temperature (<40° C). This new organic extract (B) was then solubilized in the methanol/water (90/10,330 ml/g) and partitioned with hexane (8x 125 ml/g). The hexane phase was discarded; water was added in order to have methanol /water (70/30). This phase was then partitioned with methylene chloride (6x125 ml/g of organic extract B). The methylene chloride phases were combined, concentrated under vacuum at low temperature (<40° C) and then stored in darkness, under nitrogen at 4° C.

Antibacterial assay

The Microorganisms used for the study were obtained from Department of Biotechnology, SRM Arts and Science College, Tamil Nadu, India. The antibacterial assay was determined against *Proteus vulgaris, Proteus mirabilis, Salomonella typhi, Salmonella paratyphi B, Vibrio cholerae and Staphylococcus aureus* using the paper disk method (El-Masry et al., 2000). Whatmann No.1 filter paper disk of 6-nm diameter was sterilized by autoclaving for 15 min at° C. The sterile paper discs were impregnated twice with 10µ l of different algal extracts. Agar plates were surface inoculated uniformly from the broth culture of the tested microorganism. The impregnated disks were placed on the medium suitably spaced apart and the control discs were also included on each plate containing the same solvent used for extraction or purification. In each plate the antibacterial activities of algal extracts were compared to the reference antibiotic. The plates were then incubated at 37° C for 24 hours. The diameter(mm) of the growth inhibition zone caused by the extracts of marine organisms was examined. All the assay was carried out in duplicate.

RESULTS AND DISCUSSION

Table 1 shows the result of the *in vitro* testing of extracts against pathogenic bacteria. As evident the organic extract A (Water)showed no activity against pathogenic bacteria, the organic extract B appeared (Methanol)to be the most active. The extract B exhibited weak activity against *Proteus mirabilis, Proteus vulgaris, Pseudomonas aeroginosa, Salmonella paratyphi B and Vibrio cholerae.* It exhibited no activity against *Salmonella typhi*, the fraction C appeared to inhibit *Proteus mirabilis*, *Samolnella typhi* and *Staphylococcus aureues* weakly, whereas *Vibrio cholerae* was inhibited effectively by this fraction. Extract B exhibited it maximum activity against *Staphylococcus aureus.* The extract C, (Ethanol) though inhibited only four of the seven bacteria tested, the inhibition was observed to be very effective. No solvent control produced zone of inhibition. The study confirms the antibacterial properties of *Skeltonema costatum.* The nature of the algal extract (aqueous or organic), the extraction procedures and the assay conditions may influence the result.

CONCLUSION

In conclusion, the present study suggests that *Skeletonema costatum* has anti-bactericidal activity against pathogenic bacteria. An improved knowledge of the composition, analysis, and properties of *Skeletonema costatum* with respect to antimicrobial compounds would assist in efforts for the pharmaceutical. Bactericidal activity of unsaturated and saturated long chain fatty acids have been reported by Nieman 1954, Galbraith and Miller 1973 a,b,c. They have shown that fatty acids of chain length more than 10 carbon atoms induced lysis of bacterial protoplasts. A lipophilic antibacterial substance named chlorellin produced by *Chlorella vulgaris* was reported to be an autoinhibitor of the algae when it was excreted in the culture medium Pratt, 1948. More recently an autoinhibitor a fatty acid named 15-hydroxy-eicosapentaenoic acid has also been identified in *Skeletonema costatum* Imada et al., 1992. This autoinhibitor, produced from the initial growth stage and released into the culture medium, could also have like for Chlorella vulgaris an antibacterial action. So, due to the increase of therapeutic resistance to the usual antibiotics Hjeltnes et al., 1987; Aoki, 1992; Nash et al., 1992 and due to its potential antipathogenic actions by its intra andror extra-cellular products, there appears to be a significant role for *Skeletonema costatum* in the control of Human Pathogens. Disc diffusion tests for antibiotic activity have been reported as of doubtful quantitative significance (Schneierson and Amsterdam (1959). According to Jorgensen and Nielson (1961), it was found that antibiotics in charged dried discs underwent a progressive loss in potency when kept at room temperature. We have found that freshly prepared discs when refrigerated in dark showed negligible loss in activity up to one-month storage. However, further assays need to be done, notably in vivo. First, it would be of great interest to determine if this potent antibacterial action is due to an extracellular product. Some assays using dialysis culture Cooper et al., 1983 or those combining culture supernatant and bacterial culture medium Fabregas and Veiga, 1984 could allow us to determine if an antipathogenic metabolite is excreted. Concerning the *in vivo* experimentation's, it would be interesting to test the algal powder as a food supplement for prevention or therapy of Human Pathogens.

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