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Antibacterial Activity of the Marine Diatom, *Rhizosolenia alata* (Brightwell, 1858) Against Human Pathogens

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Abstract: Cultured marine diatom, *Rhizosolenia alata* (Brightwell) were freeze dried and extracted with solvents such as acetone, chloroform, chloroform: methanol (1:1), methanol: distilled water (4:1) and distilled water. The extracts were screened against 7 human pathogenic bacteria for their antibacterial activity. All the organic solvents showed activity against all the pathogens tested with a maximum activity in chloroform against *Proteus vulgaris* and minimum in chloroform: methanol against *Staphylococcus aureus*. However, there was no activity in distilled water extracts against all the pathogens tested.

Key words: Isolation of diatom, culture, human pathogen, antibacterial activity

INTRODUCTION

Marine microalgae have been recognized as potential sources of antimicrobial substances Walter and Mahesh (2000). Austin *et al*. (1992) used micro algal powder as food supplement for prevention and treatment of bacterial fish diseases. Antibacterial activity of extracts from *Skeletonema costatum* was proved against some common pathogens in aquaculture (Naviner *et al*., 1999). However there are no reports regarding the antibacterial compounds of micro algae against human pathogens (Walter and Mahesh, 2000; Manivasaham *et al*., 1989). 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 future role of microalgal compounds in drug discovery is especially in the priority areas for development of new medicines, namely to fight viral infections and cancer and combat infections from antibiotic resistant bacteria and fungi (Katircioglu *et al*., 2004). Hence the present study aimed to estimate the antibacterial activity of a most common marine diatom, *Rhizosolenia alata* against some common human pathogens.

MATERIALS AND METHODS

The diatom, *R. alata* was isolated from the plankton sampled from Vellar estuary (11° 29' N; 79° 46' E) in January 2006, southeast coast of India by using plankton net (Cloth No. 25 with mesh size of 42 μm) and cultured in F/2 medium (Guillard, 1983) for 9 days under optimal laboratory conditions. The cells were harvested at the end of 9th day (cell density 2.6×10^5 mL^{-1}) and then freeze dried. Dried samples were extracted with different solvents viz., acetone, chloroform, chloroform: methanol (1:1), methanol: distilled water (4:1) and distilled water. Fifty milligram of dried algal powder was added with 1 mL of solvent, shaken well, kept for one hour at room temperature and centrifuged in the cold temperature for 20 min at 3000 rpm. The clear supernatant was pipetted out and stored under -4°C until use.

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In vitro antibacterial activity of diatom extracts were determined against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi* and *Vibrio cholerae* (Source: Jawaharlal Nehru Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry) using the paper disk assay method (El-Masry *et al*., 2000). Whatman No.1 filter paper disk of 6-mm diameter was sterilized by autoclaving for 15 min at 121°C. The sterile disks were impregnated with different extracts (500 μg mL^{-1}). Agar plates were surface inoculated uniformly from the both culture of the tested microorganisms. In all cases, the concentration was approximately 1.2×10^{8} CFU mL^{-1}. The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 37°C for 24 h disk of penicillin-G (P) (Himedia No. SD 028) was used as a positive control and discs with solvents (without extract) as control. The Antibacterial activity was read by noting the presence or absence of clear zones of inhibition surrounding the discs and was measured as the radial extent of the clear zone (diameter mm). All assays were carried out in duplicate.

RESULTS AND DISCUSSION

The results (Fig. 1) obtained in the present study revealed that *R. alata* has produced some antibacterial compounds against human pathogenic bacteria tested. Among the solvent extracts of *R. alata* tested, chloroform extract showed growth inhibition activity against all the seven pathogenic bacteria tested and maximum inhibitory activity was found against *P. vulgaris* with zone of clearance of 17 mm and less activity against *S. aureus* (5 mm). There was no inhibition activity found with distilled water extracts against all pathogenic bacteria tested. The overall observation inferred that maximum growth inhibition activity was found with chloroform extract against *P. vulgaris* and minimum with chloroform + methanol extract against *E. coli* (2 mm). As earlier reports of Walter and Mahesh (2000), in the present study acetone and chloroform extracts showed considerable activity against most of the test organisms. However, chloroform + methanol extracts showed only moderate activity, methanol + water fraction showed negligible activity and the water fraction revealed no activity.

The antibacterial activity of marine diatom shown in the present study was supported by the observation of Manivasagam *et al*. (1989). Their observation on antibacterial activity of the diatom *Pleurosigma elongatem* showed its growth inhibition activity against *B. subtilis*, *S. aureus*, *E. coli*, *S. typhi*, *V. cholerae* in acetone, chloroform, chloroform + methanol, methanol + water, as the observations of present study. There was no antibacterial activity was found in distilled water extracts. The difference in the antibacterial activity may be due to species specific characteristics and the efficiency of the solvents used in the extraction of antibacterial substances (Walter and Mahesh, 2000).

The results of the present study inferred that the antibacterial activity of *R. alata* may be to prevent the colonization of epiphytic bacteria on the surface of the algae. Such control and regulation of the epibiota by antibiotics diffusing outwards from the thallus of some marine planktonic algae was indicated by McN Sieburth (1964). The less activity found with methanol + water extract may be due to the inefficiency of the method and further research on the characterization of the antibacterial compounds from *R. alata* are under process. 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.

The present study is a preliminary attempt to screen marine microalgae for bioactive compounds. It appears from the present investigation that, a marine microalga, *R. alata* is potential source of novel antibacterial compounds. Characterization of the antibacterial compound and evaluation of methods employed in antibiotic assay deserve further research to give a clear picture of the antibacterial activity of the algae.

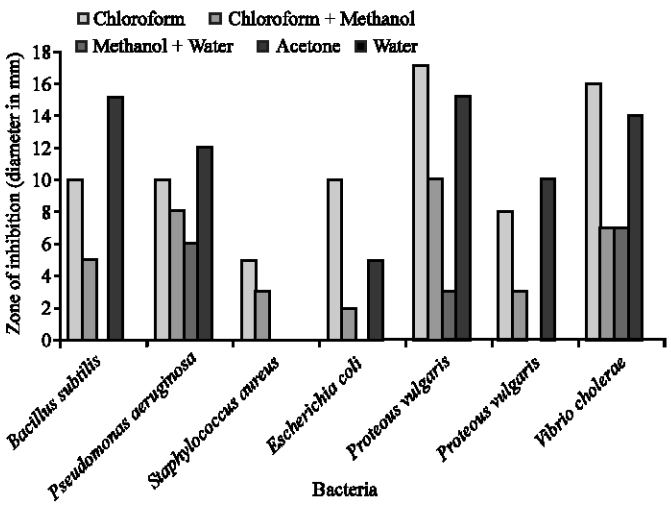

Fig. 1: Antibacterial activity of different grade solvent systems of *Rhizosolenia alata*

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REFERENCES

Austin, B., E. Baudet and M. Stobie, 1992. Inhibition of bacterial fish pathogens by *Teraselmis suecica*. J. Fish. Dis., 15: 55-61.

El-Masry, H.A., H.H. Fahmy and A.S.H. Abdelwahed, 2000. Synthesis and antimicrobial activity of some new benzimidazole derivatives. Molecules, 5: 1429-1438.

Guillard, R.R.L., 1983. Culture of Phytoplankton for Feeding Marine Invertebrates. In: Culture of Marine Invertebrates, Selected Readings. Carl J. Berg, Jr. (Ed.), Hutchinson. Ross Publishing Co. Stroudsberg. PA, pp: 108-132.

Jorgensen, E.G. and E.S. Nielsen, 1961. Effect of filtrates from cultures of unicellular algae on the growth of *Staphylococcus aureus*. Physiol. Plant., 14: 896-908.

Katircioglu, H., B.S. Akin and T. Atici, 2004. Microalgal toxin(s): Characteristics and importance. Afr. J. Biotechnol., 3: 667-674.

Manivasaham, S., R. Selvaraj, A. Purushothaman and A. Subramanian, 1989. Antibacterial activity of *Nitzschia obtusata*. Curr. Sci., 58: 83.

McN Sieburth, J., 1964. Antibacterial substances produced by Marine algae. Develop. Ind. Microbiol., 5: 24-134.

Naviner, M., J.P. Berge, P. Durand and H. Le Bris, 1999. Antibacterial activity of the marine diatom *Skeletonema costatum* against aquacultural pathogens. Aquaculture, 174: 15-24.

Schneierson, S.S. and D. Amsterdam, 1989. A simplified tube procedure for the routine determination of bacterial sensitivity to antibiotics. Am. J. Clin. Pathol., 31: 81-86.

Walter, C.S. and R. Mahesh, 2000. Antibacterial and antifungal activity of some marine diatoms in culture. Ind. J. Mar. Sci., 29: 238-242.