BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2012. Vol. **9**(1), 399-403

Bioactivities of Protein Isolated from Marine Sponge *Zygomycaleparishii*

K.V. Anitha[1], W. A. Manjusha[1]*, W.A. Anusha[1], B. V. AmrithaKrishna[1], L. Lavanya[1], J. J. Regin Prasad[1], R. A. Remya[1] and Vijayasurya[1]

[1]Malankara Catholic College, Mariagiri, Kaliakkavilai, K.K. Dist -629 153, India.

(Received: 15 January 2012; accepted: 28 February 2012)

Today, one emerging source of small molecule drug lead in the world's ocean. Based on the present findings, the marine sponge extract from *Zygomycaleparishi* have potential antimicrobial activity against pathogenic bacteria and fungi. Methanolic extract of sponge *Zygomycaleparishii*showed three bands ranging from 70-150 kDa and aqueous crude extract of *Zygomycaleparishii*revealed three bands which ranging between 66-205 kDa on SDS-PAGE.The results of present investigation revealed that,the marine sponges are a potential source of novel antibiotic leads.

Key words: Marine sponge, *Zygomycaleparishi*, Antimicrobial activity, SDS-PAGE.

The ocean is said to be the medicine chest of the future and the marine sponges plays vital role among them. The marine environment is an exceptional reservoir of bioactive natural products, which produce several novel structures with unique biological properties. Among the groups of marine organisms sponges are the most diverse and abundant, due to their soft bodies and sedentary life styles. The presence of large amounts of microorganisms within the mesohyl of many demosponges has been well documented (Hentschel *et al.* 2002; ImhoV and Stöhr 2003). Sponge mesohyl was referred as "micro-environments" providing a broad variety of ecological niches (Thiel *et al.* 2007). Bacteria can contribute up to 40% of the sponge biomass (equal to about 108 to 109 bacteria g tissue¡1) and are probably permanently associated with the host sponge unless they are disturbed by external stress factors (Friedrich *et al.* 2001; Thoms *et al.*2003; Webster and Hill 2001).Presently, occurrence or new infectious diseases and development of drug resistant pathogens are threatening the living organisms. More number of antimicrobial substances developed so far was impractical to tackle the situation, because of their complex chemical nature, (that produceenvironmental consequences) and the development of resistant pathogenic strains. So the present study is in urge, to develop ecofriendly, easily available, cheap antimicrobial compound.

MATERIALAND METHODS

Sponge was collected by scuba divers from South west coast of India. Voucher specimens were either preserved in 70% ethanol immediately upon collection or freeze-dried for chemical characterization. Freeze-dried specimens were prepared for histology by dehydration in a very weak solution of detergent for 24 h, followed by preservation in 70% ethanol. Histological

* To whom all correspondence should be addressed.
E-mail:manjusha_aj@yahoomail.co.in

sections and spicule preparations were made as in the work of Kelly-Borges and Vacelet (1995).

The sponges collected in methanol containers were squeezed in a tissue homogenizer, depending on the nature of the sponge species, which was used for extraction. In the case of *Mycale* species , they were cut into small pieces, and squeezed to prepare the crude extract using a mortar and pestle, using methanol as solvent . They were extracted thrice and the combined extract was concentrated in a rotary vacuum evaporator rotary vacuum evaporator (Buchi, Flawil, Switzerland) at room temperature. Thus concentrated crude extract was collected in air tight plastic containers and kept in refrigerator.The aqueous extract of sponge was prepared by squeezing the sand- free specimens in triple distilled water. The resultant solution was filtered and dialyzed, using dialysis membrane against D-glucose to remove the excess water. The supernatant obtained was lyophilized and stored at 4^0C in a refrigerator for further use as aqueous crude extract for SDS-PAGE.

The antimicrobial susceptibility testing was done by Kirby-Baeur disc diffusion method. This method allows the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that result from diffusion of the agent into the medium surrounding the disc. In this procedure, filter paper discs of uniform size (6mm) are impregnated with specified concentrations of two methanolic sponge extract and then placed on the surface of an agar plate that has been seeded with the organisms to be tested. The medium of choice is Mueller-Hinton agar, with pH of 7.2 to 7.4, which is poured into plates to uniform depth of 5mm and refrigerated on solidification. Label the covers of each plates with the name of test organisms to be inoculated ie., *Acetobactersp*(MTCC2903),*Bacillus cereus* .(MTCC430), *Bacillus subtilis* (MTCC441) , *Gluconobacteroxydans*(MTCC904), *Pseudomonas aeruginosa* (MTCC741) obtained from Microbial Type Culture Collections (MTCC), Chandigarh, India. Bacterial colonies were allowed to grow overnight at 37^0C , then the inhibition zone around the disc was measured (Cappucino*et al*., 2004).

Petri dishes with Rose Bengal Agar medium were inoculated *Aspergillusaculeatus* (MTCC1331), *Aspergillusniger*(MTCC281), *Candida tropicalis* (MTCC1000),. *Rhizom ucormicchii* (MTCC546) obtained from Microbial Type Culture Collections (MTCC), Chandigarh, India. Round paper discs of 6mm were dipped in to 0.001 mL of each methanolic sponge extract and placed in the centre of the inoculated petri dishes. Fungal colonies were allowed to grow overnight at 20ºC and then the inhibition zone around the disc was measured.

The crude extracts were purified by ammonium sulphate precipitation. During ammonium sulphate precipitation, the salt has to be prevent increase of high local concentration. Ammonium sulphate was used for precipitation of total proteins at -90% saturation or for precipitation of proteins using different saturations of salt 40% saturation was done to precipitate the proteins from the culture. The solution was equilibrated for approximately one day in cold condition to ensure complete precipitation and then the precipitate was collected by centrifugation and it was further purified by dialysis method using dialysis membrane. Thus prepared were used for One dimension Sodium Dodecyl Sulphate (SDS) polyacrylamide Gel Electrophoresis (PAGE) was carried out. The protein content of methanolic crude extract and aqueous crude extract were dissolved in 300µl of sample buffer and the samples were loaded into several lanes of 30% gradient gel along with the molecular weight standards. The process adapted a power supply of 80 volts for 3 hours. After electrophoresis, the gel was stained with silver staining (Laemmli, 1970).

RESULTS AND DISSCUSSION

Present study revealed that the tested in marine sponge *Zygomycaleparishi* possessed potential antibacterial activity against *Acetobactersp, Bacillus cereus, B. subtilis*, *Glucanobactoroxydens*,*Pseudomonas aeruginosa* (Fig.1). When tested by the disc diffusion method, methanolic sponge extract of *Zygomycaleparishi* showed significant activity against *B. subtilis* and *Glucanobactoroxydens* produced minimum inhibitory activity 19mm. Among the five species of bacteria sponge methanolic extract showing highly significant antibacterial activity against *P. aeruginosa*

In antifungal activity it was found that the metanolic extract successfully prevent the

growth of fungi (Fig. 2). The inhibitory zone produced by extract against *Aspergillusaculeatus* 12mm ,*Aspergillusniger*is 13mm, *Candida tropicalis*is 9mm and*Rhizomucormicchii* is 14mm. Among this *Zygomycaleparishii*has more activity against *Rhizomucormicchii*(14mm) followed by *Aspergillusniger (*13mm*) and Candida tropicalis* (9mm*)*. The extract shows feeble activity against*Aspergillusaculeatus* (12mm*).*

Fig.2. Antifungal activity of *zygomycaleparishi* against fungi

In the present study, sponges *ZygomycaleParishi*collected from Vizhijam coastal area, produced potent antimicrobial extracellular products. This property indicates that the species might have defense mechanisms in the host sponge. These sponges has extreme potent of antifungal and antibacterial activity (Chelossi*et al*., 2006). Bergquist and Bedford (1978) have been reported that the antibacterial agents produced by sponges may have a role in enhancing the efficiency with which sponge retain bacterial food and also reported that the activity was higher in temperate species than tropical species (87% as opposed to 58%) and the sponge extract more frequently inhibited the growth of marine bacteria. extracts of different sponge species show activity on various microorganisms, the obtained results suggest that some sponges are seemed to be pathogen specific, which are active against the bacteria (*Clathria*sp., *Sigmodocia*sp., *Callyspongia*sp.) and some are against the fungi (*Callyspongia*sp., *Zygomycale*sp.)(Rajagopal*et al*.,2008). Faulkner *et al.* (2001) have stated that marine sponges have a potential to provide future drugs against important diseases, such as malaria, cancer and a range of viral diseases. Rifai*et al.* (2004) isolated Untenospongin B from the marine sponge *Hippospongiacommunis*and tested for its antimicrobial activity against bacteria and human pathogenic fungi using agar disk method and found to possess a broad and strong activity toward the test organisms.

SDS-PAGE analysis revealed the presence of bands in them. Methanolic extract of sponge*Zygomycaleparishii*showed three bands ranging from 70-150 kDa. Aqueous crude extract of *Zygomycaleparishii*revealed three bands which ranging between 66-205 kDa. It indicates that the fractions might have been responsible for the potent antimicrobial activity(Fig. 3).

Selvin and Lipton (2004) and Kanagasabhapathy*etal.* (2004) have also confirmed that the sponge species of the southern

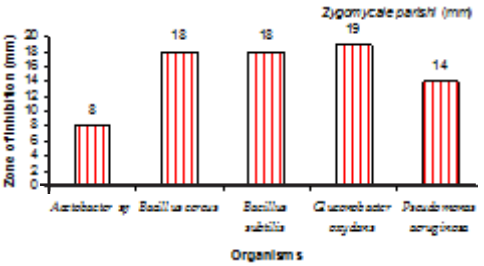

Fig. 1. Antibacterial activity of *Z .parishi* against bacteria.

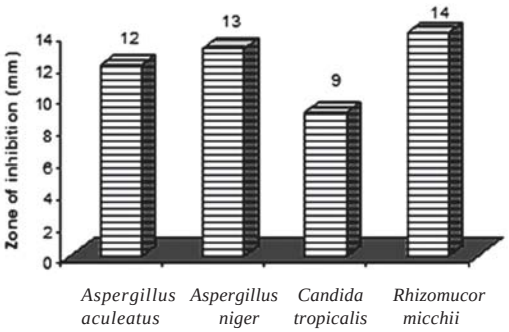

Fig. 2. Antifungal activity of *Zygomycaleparishi* against fungi

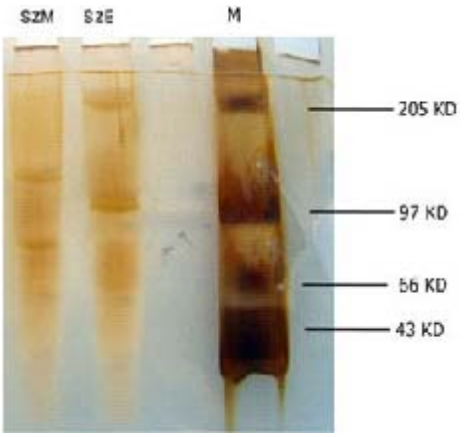

Fig. 3. Protein profiling of *Z. parishi*with two solvent extracts (Lane1-Aqueous and Lane2-methanol,M-Marker) by SDS-PAGE analysis.

Eastern Peninsular Indian Coast are the ideal candidates for the production of various antimicrobial (bacterial and fungal) and antifouling drugs. In the present study, the SDS-PAGE on the 30% gradient gel, the crude protein toxins yielded 3 bands each in the methanolic extract and aqueous crude extract of *Z .parishi.* The secondary metabolites metabolites of host sponge *D. nigra* have demonstrated broad spectrum antibacterial activity and inhibited the growth of all tested bacteria (Selvin*et al*.,2004a). Culture-based studies indicated that the supplementation of host sponge extract in the culture media drastically increased the number of morphotypes (Selvin*et al.,2004b)*. Sponge symbionts are thought to benefit their hosts in many ways (Wang, 2006;Taylor *et al.*, 2007). This study revealed the presence of potentially new bacterial phylotypes in *M. armata*(Wang *et al*.,2008). Based on the findings, it could be inferred that sponges could form a reliable source for bioprospecting of next generation pharmaceutical agents.

REFERENCES

1. Adriana, S., ThomazVieiralves, Flavia R.M. Lamarao, Leonardo L.M. Assumpcao, Debor Gomes, LiaJascone Ana LuizaValadao, Rodolpho M. Albano, Gisele Lobo-Hajdu. Field preservation and optimization of a DNA extraction method for Porifera. *Porifera Research: Biodiversity, Innovation and Sustainability*. 555-560, (2007).
2. Bergquist, P.R. and Bedford, J.J. The incidence of antibacterial activity in marine Demospngie, Systemic and Geographic consideration. *M ar. Biol*. **46**: 215-221, (1978)
3. Cappucino, Natalie Sherman. The Kirby –Bauer Antibiotic Sensitivity Test. Microbiology, A laboratory manual. Sixth edition. 264-266, (2004).
4. Chelossi, E., Mancini, I., Sepcic, K., Turk, T., Faimali, M. Comparitive antibacterial activity of polymeric 3-alkylpyridinium salts isolated from the Mediterranean sponge Renierasarai and their synthetic analogues. *Biomol.Eng.* **23**: 317-323, (2006).
5. Faulkner, A.D., Wright, G., Matthee, F. and Konig, G.M. Culture extracts of sponges showed antifungal, antialgal and antibacterial activity and cytotoxicity. *Mycological Research.* (2001).
6. Friedrich A.B, Hacker J, Fischer I, Proksch P, Hentschel U. Temporal vaiations of the microbial community associated with the Mediterranean sponge *Aplysinaaerophoba*. *FEMS MicrobiolEcol,* **38**: 105–113, (2001).
7. Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS. Molecular evidence for a uniform microbial community in sponges from diVerent oceans. *Appl Environ Microbiol*, **68**: 4431–4440, (2002).
8. ImhoV JF, Stöhr R. Sponge-associated bacteria, general overview and special aspects of bacteria associated with *Holichondriapanacea*. In: Müller WEG (ed) Molecular marine biology of sponges. *Springer*, Berlin, (2003).
9. Kanagasabhapathy, M., Nagata, K., Fujita, Y., Tamura, T., Okamura, H. and Nagata, S. Antibacterial activity of the marine sponge *Psammaplysillapurpurea*: Importance of its surface-associated bacteria. Proceedings of Oceans'04. *Techno-Ocean*, **4**: 1323-1329, (2004).
10. Laemmli UK."Cleavage of structural proteins during the assembly of the head of bacteriophage T4". *Nature.* **227**(5259) : 680–685, (1970).
11. Liu, P.V. Survey of hemolysin production among species of *Pseudomonas*. *J. Bacteriol*. **74**: 718–727, (1957).
12. Mitra, A., Santra, S.C. and Mukharjee, J. Distribution of actinomycetes, their antagonistic behavior and the Physio-chemical characteristic of the worlds largest tidal mangrove forest. *Applied Microbial Biotchnol*., **80**: 685-695, (2008).
13. Rajagopal, B., S.R. Jegan, R.V. Seena, S. Suma, T.K. Angiesh and Baby Joseph. Bioactive screening of Peninsular Indian Marine sponges on selected microorganisms. *Journal of basic and applied biology*. **2**: 56-64, (2008).
14. Rifai, S., Fassouane, A., Kijjoa, A. and Soest, R.V. Antimicrobial activity of untenospongin B, a metabolite fro the marine sponge *Hippospongiacommunis* collected form the Atlantic coast of Morocco. *Marine Drugs,* **2**: 147–153, (2004).
15. Saadoun, I. and A. Muhana, Optimal production condition, extraction, partial purification and characterization of inhibitory compound(s) produced by *Streptomyces* Ds-104 isolate against Multi-drug resistant *Candia albicana*. *Curr. Trends. Biotechnol. Pharm*. **2**: 402-420, (2008).
16. Samuel, M., Lu, M., Pachuk, C.J ,Satishchandran, C. A spectrophotometric method to quantify linear DNA. *AnalBiochem.* **313**: 301-306, (2003).
17. Schwartsmann, G., Da Rocha, A.B., Mattei, J., Lopes, R. Marine- derived anticancer agents in clinical trials. *Expert OpinInvestig Drugs*. **12**:

1367-1383, (2003).

18. Selvin, J. and Lipton, A.P. Biopotentials of secondary metabolites isolated from marine sponges. *Hydrobiologia,* **513**: 231–238, (2004).
19. Selvin,J , Soniya Joseph, Asha, Manjusha, Sangeetha,Jayaseem, Antony, DenslinVinitha. (2004) Antibacterial potential of antagonistic *Streptomyces* sp. isolated from marine sponge *Dendrillanigra* .*FEMS Microbiology Ecology*, **50**(2): 117–122, (2004).
20. Selvin. J. and Lipton, A.P. Antimicrobial activities of marine sponges collected off the coast of Santa Catarina – Biopotentials of secondary metabolites. *Hydrobiologia,* **513**: 231-238, (2004).
21. Taylor MW, Radax R, Steger D, Wagner M. (2007). Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microb Mol Biol Rev*, **71**: 295–347, (2007).
22. Thiel V, Neulinger SC, Staufenberger T, Schmaljohann R, ImhoV JF. Spatial distribution of sponge-associated bacteria in the Mediterranean sponge *Tethyaaurantium*. *FEMS MicrobiolEcol* , **59**: 47–63 (2007).
23. Thoms C, Horn M, Wagner W, Hentschel U, Proksch P. Monitoring microbial diversity and natural products proWles of the sponge *Aplysinacavernicola*following trasplantation. *Mar Biol*, **142**: 685–692, (2003).
24. Wang G. Diversity and biotechnological potential of the sponge-associated microbial consortia. *J IndMicrobiol Biotechnol,* **33**: 545–551, (2006).
25. Wang,G, Sang-Hwal Yoon and Emilie Lefait Microbial communities associated with the invasive Hawaiian sponge *Mycale armata The ISME Journal,* **3**: 374–377, (2009).
26. Webster N, Hill RT. Theculturable microbial community of the Great Barrier Reef sponge *Rhopaloeidesodorabile*is dominated by an a-Proteobacterium. *Mar Biol*, **138**: 843–851, (2001)