Antimicrobial potential of marine sponge *Ircinia fasciculate* from south peninsular coast of India

Priya[1*], L., Maria Saroja[2], M. and Huxley[1*], V. A. J.
[1] P.G and Research Department of Zoology, Thiru. Vi. Ka. Govt. Arts College, Thiruvarur, Tamilnadu, South India.
[2] St. Ignatius College of Education, Palayamkottai, Tamilnadu, South India

Abstract : *In vitro* antimicrobial screening of marine sponge *Ircinia fasciculate* collected from south peninsular coast of India, against selected bacteria and fungi was conducted in this study. Crude sponge extracts of the marine organism *Ircinia fasciculate* demonstrated activity against microbes tested. The extracts showing good antimicrobial activity are undergoing further analysis to identify the active constituents.

Key words: *In vitro* antimicrobial activity, *In vitro* antifungal activity, Marine sponges,

I. INTRODUCTION

Drug discovery from marine sponges have been considered as a very fertile field for the past decades with respect to the diversity of their primary and secondary chemical components and metabolites (Perdicaris *et al.*, 2013). It was proved that marine sponges produce an enormous array of antitumor, antiviral, anti-inflammatory, immunosuppressive, antibiotic, and other bioactive molecules that have the potential for therapeutic use. More than 15,000 marine products have been isolated and tested until the last 20 years until 2012. Sponges have been the champion producers with large diversity of natural components. Screening of organic extracts from marine sponges and other marine organisms is a common approach to identify compounds of biomedical importance (Samuel, 2015). The bioactive substances from sponges the terpenes, sterols, cyclic peptides, alkaloids, fatty acids, peroxides and amino acid derivatives have been described from their associated microorganism (Sfanos *et al.*, 2005). Bioactive compounds from marine sponges have extensive use in the treatment of many diseases and these compounds act as the templates for synthetic modification. Several molecules isolated from various sponges are currently involved in the advanced stage of clinical trials. The demospongiae, *Ircinia fasciculate* (Thomas, 1985) is a common sponge on the intertidal rocky shores of south peninsular coast of India. They are a rich source of structurally novel and biologically active metabolites. From the sponge *Ircinia fasciculate*, the biologically active molecules show strong antibiotic, analgesic and anti-inflammatory properties (Rajeevkumar and Xuzirong, 2004). The discovery of new classes of antibiotics is necessary due to the increased incidence of multiple resistances among pathogenic microorganisms to drugs that are currently in clinical use (Fenical, 1983). This study describes the screening of crude extracts of marine sponge *Ircinia fasciculate*, collected from the intertidal rocky shores of South peninsular coast of India for antibacterial and antifungal activities.

II. MATERIALS AND METHODS

Collection of sponges

Specimens of the marine sponge *I. fasciculate* (Brownish yellow sponge) were collected from the peninsular coast of India, especially Arokiapuram coast which is located about 6 km from Kanyakumari (Lat8° 4'N; Long 77° 50'E) to Vattakottai road (Lat 8°3'N; Long 77° 05'E) south east coastal region of TamilNadu, India.. An eco-friendly bulk collection of the sponges by bycatch was carried out during November and December, and April and August and taken-up for isolation and bioactivity screening of the secondary metabolites.

Preparation of crude extracts from sponges

Sponges were washed lightly with water and extracted with 200 ml methanol, Chlroform, Acetone and Hexane for about 15-20 days. Solvents were removed by rotary vacuum evaporator (Buchi type) under reduced pressure so as to get the crude sponge extract. The concentrated crude extract was collected in airtight glass vials and kept in the refrigerator for further use.

III. ANTIBACTERIAL ACTIVITY

The antibacterial activities of the extracts of the *I. fasciculate* sponge were determined by the standard agar disk diffusion assay Perez *et al.*, (1990) using Muller Hinton agar (Hi Media). Bacterial isolates were obtained from Biotech Research Laboratory, Dept. of Zoology, Thiru. Vi. Ka. Govt. Arts College, Tiruvarur. Plates to Muller Hinton Agar (MHA) are marked with names, dates and microorganism to be tested. Cotton swab dipped in a suspension culture sterile test, and then cotton sticks played on the tube wall to liquid from dripping from the cotton section. Spread the entire surface of the plate, to obtain equitable growth, cotton sticks smeared horizontally. Plate to be left to dry for approximately 5 minutes, then place a paper disc that has been soaked with the sample that was tested on plate agar surface. Paper disc pressed with tweezers; need not be too hard because it will damage the surface of the agar. Discs of Streptomycin (25µg/ml) was used as positive control. Plates already affixed to the paper discs were incubated at optimal growth temperature of pathogenic bacteria that were tested. Having already tested bacteria grow evenly and the clear zone surface were noted.

IV. ANTIFUNGAL ACTIVITY

Antifungal activity of the crude extract of marine sponge *I. fasciculate* was determined by using the standard method (Selvin and Lipton, 2004). Fungal Isolates were obtained from, Biotech Research Laboratory, Dept. of Zoology, Thiru. Vi. Ka. Govt. Arts College, Tiruvarur. The fungal cultures were maintained in 0.2% dextrose medium at 5.6 pH and the optical density 0.10 at 530 nm was adjusted using spectrophotometer. Each fungal inoculum was applied on plate and evenly spread on potato dextrose agar using a sterile swab, then place a paper disc that has been soaked with the sample that was tested on plate agar surface. Discs of the Fluconazole was used as the positive control. Agar diffusion assay was followed to evaluate the antimicrobial activity. The Petri plates were incubated at 30°C for 2 days. At the end of the 48 hrs, inhibition zones formed in the medium were measured in millimetres.

V. RESULT

Table 1 shows the result of the *in vitro* testing of sponge extracts against pathogenic bacteria. This can be observed from the emergence of clear zone around the paper disc. Clear zone around the paper disc indicates that the absence of bacteria that can grow after incubation due to the antibacterial compounds in the area. Extract of sponge *I. fasciculate* have no antibacterial activity against *E.coli*. Methanol extract of sponges showing good activity towards *Staphylococcus aureus*, but chloroform extract showing weak activity, while acetone and hexane extract is inactive. However Methanol, Chloroform and Acetone extract showing weak activity against *Salmonella typhi*, while Hexane extract is inactive.

Table 1: Antibacterial activity of crude extract of marine sponge organism *Ircinia fasciculate*

Organic solvent	Bacteria			
	Escherichia coli	*Staphylococcus aureus*	*Salmonella typhi*	*Bacillus subtilis*
Methanol	-	++	+	++
Chloroform	-	+	+	-
Acetone	-	-	+	-
Hexane	-	-	-	-

(-) No activity, (+) weak activity (7–8mm halo), (++) good activity 9–12-mm halo).

Also Methanol extract was showing good activity against Bacillus but Methanol, Chloroform and Actone extract is inactive. In case of antifungal activities, Methanol and Acetone extract of sponge showing good activity against *Aspergillus sp*.but Chloroform and Hexane extract are inactive. Methanol, Acetone and Chloroform extract showing good activity against *Penicillium sp*. except Hexane extract, which was inactive. Also Chloroform and Acetone extract was weakly active againt *Fugarium sp*. while Methanol and Hexane was inactive. However, each extract was inactive against Alternaria sp.

Table 2: Antifungal activity of crude extract of marine sponge *Ircinia fasciculate*

Organic solvent	Fungi			
	Aspergillus sp.	*Penicillium sp.*	*Alternaria sp.*	*Fugarium sp.*
Methanol	++	++	-	+
Chloroform	-	++	-	+
Acetone	++	++	-	+
Hexane	-	-	-	-

(-) No activity, (+) weak activity (7–8-mm halo), (++) good activity (9–12-mm halo).

VI. DISCUSSION

In an earlier study (Amade *et al.*, 1987), out of the 7 species of Brittany sponges, only two (*D. fragilis* and *Phakellia ventilabrum*) showed slight inhibition on some bacteria and fungi. However, in the present study, *D. fragilis* showed the inhibition against *C. albicans*, *Cryptococcus sp. A. fumicatus* and *A. niger*. Antimicrobial activities of S. officinalis, Crambe crambe and Ircinia fasciculate have also been studied (Amade et al., 1987; Burkholder and Ruetzler, 1969; Uriz et al., 1992). In this regard, present study is significant in that the antifungal activity of *S. officinalis* var. ceylonensis was higher than that of the earlier reports.

Ethanolic extracts of 19 species of sponges collected from Polynesia were tested against bacteria and fungi. Among these, 8 had no activity, 4 had very weak activity and 7 showed significant activities against bacteria and fungi (Amade *et al.*, 1982). In the present study, out of the ethanolic extracts of 9 species of sponges collected from the Palk Bay region, only 4 species (*S. officinalis* var. ceylonensis, *P. purpurea*, *D. fragilis* and *H. cribirformis*) extracts were slightly active against fungi and 5 species (*H. tenuiramosa*, *D. anchorata*, *S. inconstans* and *S. inconstans* var. digitata) extracts showed no fungal inhibition. Thus, the antimicrobial activity of sponges may vary from species to species as determined by the biochemical and physiological synthesis of antimicrobial compounds. When the chemical defense and anti-fouling activity were analysed, strong antimicrobial activity was found in the dichloromethane extract of *Ircinia spinosula* (against marine fungi and bacteria) and the ethanol extract of *Ircinia oros* (against diatoms) (Tsoukatou *et al.*, 2002). Such studies including the present one may thus be useful in the prevention and/or control of biofilm formation of microbes.

Though petroleum ether, chloroform and methanol extracts of the sponge *Tethya sp*. were tested, only the petroleum ether extract was very active against mosquito larvae. But the petroleum ether extract showed lesser haemolytic activity whereas the chloroform extract showed maximum lytic activity, indicating the presence of toxicity in the chloroform extract (Huxley *et al.*,

2014). In the present study, in the chloroform extracts activity was noticed only against *C. albicans*, *A. niger* and *Cryptococcus sp*. But the acetone extracts of the 9 species of sponges had no activity against all the fungal species tested. Of the different species of sponges, only 4 species viz *P. purpurea*, *S. officinalis* var. ceylonensis, *H. cribriformis* and *D. fragilis* exhibited inhibitory activity against the pathogenic fungi viz *C. albicans*, *A. niger*, *Cryptococcus sp. A. fumigatus* and *A. flavus* and 5 species namely *S. inconstans* var. digitata, *S. inconstans*, *C. diffusa*, *D. anchorata* and *H. tenuiramosa* showed no activity against all the fungal pathogens tested in the present investigation.

Thus, it can be inferred that the fungi are more resistant to the sponge extracts. This could be attributed to the fact that the cell walls of the fungi are composed of chitin, a nitrogen containing polysaccharide. The hard cover of the crabs and exoskeletons of arthropods are also composed of this substance chitin, which is relatively resistant, including for microbial decomposition.That view was supported by the finding that all sponge extracts had the capability to inhibit at least one strain of pathogenic microorganism (Table1). Sponges collected from the Caribbean and Tunisian Sea regions (Galeano and Martínez 2007, Touati et al., 2007) that were extracted by intermediate polar solvents, such as chloroform and ethyl acetate, inhibited both gram-positive and gram-negative bacteria. In contrast, sponge extracts from our study were moderately active against only gram positive bacteria. These results led us to conclude that the extracts of Andaman Sea sponges do not contain broad-spectrum antimicrobial substances, and further more, that the cell walls of gram-positive bacteria are more highly sensitive than gram-negative bacteria to attack by antimicrobial agents, because of the teichoic acid assembly in their structure (Duguid 1965). However, some extracts from specific taxa, including the DCM part of *Chondrosia reticulata* and the hexane part of *Axinyssa sp.* (LAN-06-21), showed highly potent activity against *M. luteus*, with zones of inhibition about 22 and18 mm, respectively. This could have been because of the presence of long-chainanti bacterial fatty acids, particularly 8, 10,Me 2-16:0 (Nechev 2002, Rodkina 2005) inthe genus Chondrosia spp., and strong antibacterial germacranes sesqui terpene in the genus *Axinyssa sp*. (Satitpatipan and Suwanborirux 2004). The DCM extract of *Phakellia ventilabrum* showed both strong cytotoxic activity against Vero cell and moderately anti-infection activity, which reflected the presence of typical cytotoxic cyclic peptides in the extract (Pettit *et al.,* 1994, Li *et al.,* 2003, Pettit and Tan 2005).

These difference in activities due to diverse chemistry of bioactive compounds in the same sponge. Sponges are primitive marine invertebrates which contain more natural products than any other marine phylum. Many of their products have strong bioactivities including anticancer, antimicrobial, larvicidal, hemolytic and anti-inflammatory activities and are often applicable for medical use (Andersson, 2003).

VII. ACKNOWLEDGEMENTS

Authors are thankful to Dr. G. Geetha, Principal, TVKCAS, Tiruvarur for providing space for this research. This is the part of Ph. D work of Mrs. L. Priya

REFERENCES

[1] Amade P, Chevolot L (1982) Antimicrobial activities of marine sponges from French Polynesia and Brittany. Mar Biol 70: 223-228

[2] Amade P, Cherroin C, Baby C, Vacelet J (1987) Antimicrobial activities of marine sponges from the Mediterranean Sea. Mar Biol 94:271-275

[3] Andersson, D. Persistence of antibiotic resistant bacteria, Curr, Opin, Microbiol., 2003;6: 452-456.

[4] Burkholder PR, Riitzler K (1969) Antimicrobial activity of some marine sponges. Nature 222:983-984

[5] Duguid JP (1965) The bacterial cell wall. Journal of Clinical Pathology 18, 258-259.

[6] Fenical W, Chemical studies of marine bacteria: developing a new resource. Chem Rev 1993;93: 1673– 1983.

[7] Galeano E, Martínez A (2007) Antimicrobial activity of marine sponges from Urabá Gulf, Colombian Caribbean region. Journal of de Mycologie Médicale 17, 21-24.

[8] Huxley, V.A.J., Simple Lotus and Sadeesnakumari (2014). Evaluation of Apoptosis Regulating Efficacy of Chocen Marine Sponge Extracts. *Journal of Theoretical and Experimental Biology*. Vol. 2(3), 10-21

[9] Li WL, Yi YH, Wu HM, Xu QZ, Tang HF, Zhou DZ, Lin HW, Wang ZH (2003) Isolation and structure of the cytotoxic cycloheptapeptide phakellistatin 13. Journal of Natural Products 66, 146-148.

[10] Nechev J, Christie WW, Robaina R, de Diego FM, Ivanova A, Popov S, Stefanov K (2002) Chemical composition of the sponges Chondrosia reniformis from the Canary islands. Hydrobiologia 489, 91-98Perdicaris et al., 2013

[11] Perez, C., M. Paul and P. Bezique. An antibiotic assay by the agar well diffusion method, Alta Biomed. Group Experiences, 1990;15: 113.

[12] Perdicaris, S,, Vlachogianni, T and Valavanidis A. 2013, "Bioactive Natural Substances from Marine Sponges: New Developments and Prospects for Future Pharmaceuticals", Nat Prod Chem Res, 1 (115), doi: 10.41/2329-6836.

[13] Pettit GR, Tan R, Herald DL, Cerny RL, Williams MD (1994) Antineoplastic agents 277 isolation and structure of phakellistatin 3 and isophakellistatin 3 from a Republic of Comoros marine sponge. Journal of Organic Chemistry 59, 1593-1595.

[14] Pettit GR, Tan R (2005) Isolation and structure of phakellistatin 14 from the Western Pacific marine sponge Phakellia sp. Journal of Natural Producs 68, 60-63.

[15] Rajeevkumar, J. and S. Xuzirong. Biomedical compounds from marine organisms. Marine Drugs., 2004;2: 123-146.

[16] Rodkina SA (2005) Fatty acid and other lipid of marine sponge. Russian Journal of Marine Biology 31, 49-60.
[17] Selvin, J. and A.P. Lipton. Biopotentials of secondary metabolites isolated from marine sponges. Hydrobiologia, 2004;513: 231-238.
[18] Sfanos, K., D.Harmody, P.Dang, A.Ledger, Pomponi,S., McCarthy, P. and Lopez, J. A molecular systematic survey of cultured microbial associates of deep-water marine invertebrates. Syst. Appl.Microbiol., 2005; 28: 242-264
[19] Thomas PA.. Demospongiae of the Gulf of Mannar and Palk Bay, Recent Advances in Marine Biology (1985) 82-86.
[20] Touati I, Chaieb K, Bakhrouf A, Gaddour K (2007) Screening of antimicrobial activity of marine sponges extracts collected from Tunisian coast. Journal de Mycologie Médicale 17, 183- 187.
[21] Tsoukatou M, Hellio C, Vagias C, Harvala C, RoussisV (2002) Chemical defense and antifouling activity ofthree Mediterranean sponges of the genus Ircinia.ZNaturforsch 57C, 161–171
[22] Uriz, M.J., D. Rosell and M. Maldonado, 1992. Parasitism, commensalism or mutualism? The case of Scyphozoa (Coronatae) and horny sponges. Marine Ecology – Progress Series 81: 247–255.