International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 2(3): May-June 2012

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Marine Sciences **Research Article……!!!**

Received: 10-05-2012; Revised; Accepted: 16-05-2012

ANTIBACTERIAL ACTIVITY OF MARINE ASCIDIAN *POLYCLINUM MADRASENSIS* (SEBASTIAN, 1952) AGAINST HUMAN CLINICAL ISOLATES

A Selva Prabhu*, G. Ananthan, R. Sathish Kumar

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502**,** Tamil Nadu, India.

Keywords:

Antibacterial activity; *Polyclinum madrasensis*; disc diffusion method; human pathogens

For Correspondence:

Selva Prabhu A

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502**,** Tamil Nadu, India.

E-mail:

antonyrajprabhu@gmail.com

ABSTRACT

Antibacterial activity of methanol and ethanol extracts of the ascidian, *Polyclinum madrasensis* was evaluated by disc diffusion method against various human pathogen isolates. Two different concentrations (0.5and 1mg/ml) were analyzed. The crude methanol extract was more active exhibiting a broad-spectrum antibacterial activity than the ethanol extract against the pathogenic microbes tested. Maximum inhibition zone (10 mm) was observed against *Escherichia coli* in 1 mg ml^{-1} concentration crude methanol extract. The inhibition zone was 3 mm in *Pseudomonas aeuroginosa* for ethanol extract of 0.5 mg ml^{-1} concentration. The ranges of inhibition zone in the tested extracts were lesser than the standard antibiotics used in all the strains. The MIC and MBC for methanolic extract tested in study inferred that the values range between 0.72-0.97 mg/ml and 0.90 -1.2 mg/ml respectively.

INTRODUCTION

Ocean has a number of organisms which are having potent bioactive compounds and are currently used as medicine. A large proportion of natural compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and mollusks and some of them are currently used in clinical trials (Proksch *et al*., 2002). Ascidians are marine invertebrates which ranks second with promising the source of drugs (Azumi *et al*., 1990). Most of the ascidians are utilized as food in various countries and they are known to produce bioactive metabolites which prevent bio-fouling and this can be considered as a kind of autogenic protection. This mechanism has proved timely to be an alternative natural medicine to human beings. From tunicates (ascidian) Tridemnum solidum, was the first marine compound entered into human cancer clinical trial as a purified natural product, but was unsuccessful in further trials (Davidson, 1993). Already various ascidians such as *Botryllus* sp., and *Didemnum* sp. were reported for producing anti cancer drugs. Halocyamine A, an antimicrobial substance was isolated from haemocytes of the solitary ascidians *Halocynthia roretzi* (Azumi *et al*.,1990). Such potential ascidians should to be explored for the pharmaceutical purpose. Tunicates have been reported as rich sources of biologically active compounds and ranked third for their overall activities, next to sponges and bryozoans (Davis & Bremner, 1999).

Although research on bioactive compounds from ascidians was recently initiated, a significant drug, Didemnin B has entered into human clinical trials. Cytotoxicity of the ascidian metabolite is the most frequently listed agent against a variety of tumor cell lines, followed by antimicrobial, antiviral and anti-inflammatory activities (Davidson, 1993). Since the early days of marine natural product discovery, Porifera (sponges) and Chordata (including ascidians) have dominated as the major contributing phyla of novel bioactive compounds (Blunt *et al.,* 2007). A number of bioactive compounds have also been isolated from ascidians, exhibiting activities such as antiviral (Rinehart *et al.,* 1984), cytotoxic (Guyot, 1989), antibacterial (Azumi *et al.,* 1990), and enzyme inhibitory activities (Sato *et al.,* 1998). These compounds are mainly comprised of various derivatives of alkaloids and peptides. There are few examples of marine derived compounds which have successfully reached the market as therapeutic drugs.

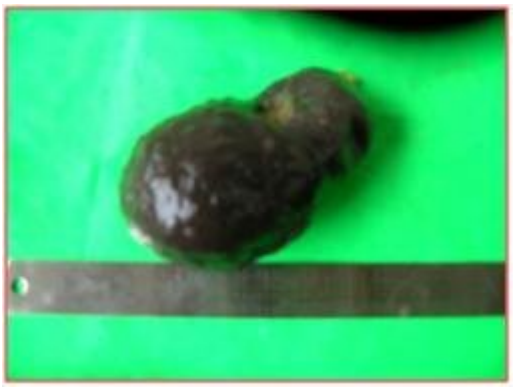
A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from plants and animals during the past two decades. The evolution of antibiotic-resistant pathogenic bacteria has stimulated the search for alternative antimicrobial agents from alternative sources including sources from the ocean. Powers of marine organisms have been realized for thousands of years and their potential as producers of pharmaceutical products have been reviewed (Baker, 2004).

MATERIALS AND METHOD

Collection and preparation of samples:

The ascidian, *Polyclinum madrasensis* (Sebastian, 1952) were collected during the low tide of the intertidal area at Tuticorin Harbor area, southeast coast of India, during April 2011. The collected samples were rinsed with sterile seawater to remove associated debris and salt. Ten gram of the samples were weighed and preserved separately in methanol, ethanol mixture (1:2) and brought to the laboratory.

***P. madrasensis* (**Sebastian, 1952)



Phylum	:	Chordata
Sub phylum	:	Urochordata
Class	:	Ascidiacea
Order	:	Enterogona
Sub order	:	Aplousobranchia
Family	:	Polyclinidae
Genus	:	***Polyclinum***
Species	:	***P. madrasensis***

Description of the species:

The colony was brown or yellowish brown in living condition. Colony mostly attached in intertidal, hull of ships, barge, pipeline and pillars. Distributed from 3 to 12m depth. It differs from other genera of family Polyclinidae by the following characters. No longitudinal folds in stomach, branchial lobes six, ovary in post abdomen, abdomen and post abdomen separated by constriction, gut loop twisted. The test is usually soft in preservative. Colonies are cushions to about 6 cm in diameter and up to 1.5 cm thick. Test gelatinous, translucent internally. Colonies are black in preservative. No sands embedded in the surface of the test. Zooids are long. Atrial lip long originated from the body wall anterior to the atrial opening. There are 12-14 rows of up to 14 relatively short oval stigmata.

Antibacterial assay:

The bioassay was carried out using the agar disc diffusion method (Baur *et al.*, 1966).Muller Hinton agar plates are prepared by pouring 15 ml of medium and allowed to solidify. The petri plates are swabbed with 24h old culture of the four selected bacterial strains. The sterile paper discs were loaded with different solvents, concentration and allowed to dry thoroughly. Then the discs were placed over the plates and incubated for 24h at 37ºC.

Minimum Inhibitory Concentration (MIC): Minimum inhibitory concentration was determined by the following procedure (Collins *et al*., 1995).

Minimum Bactericidal Concentration (MBC): Minimum bactericidal concentration was experimented after the MIC in freshly prepared agar plates, followed by standard method of Alade and Irobi (1993).

RESULTS AND DISCUSSION

The results of antibacterial activity of the crude methanol and ethanol extract of *P. madrasensis* against different gram negative clinical isolates are given in Table 1. Methanol extract at 1mg/ml concentration produced a maximum inhibition zone of 10 mm against *E. coli* and the minimum of 3 mm in *P. aeroginosa.* The corresponding zones of ethanol extract produced 10 mm and 3 mm against *E. coli* and *P. aeruginosa* respectively. Both extracts in two different concentrations showed minimum activity

against *P. aeruginosa,* whereas minimum activity was observed in the same strain at a concentration of 0.5mg/ml. Both extracts showed a broad spectrum antibacterial activity against *E. coli* followed by *K. pneumoniae, S.aureas* and *P.aeruginosa* in all the two concentrations. This observation is consistent with the findings of Ananthan *et al.* (2011, a) who reported that both methanol and ethyl acetate extract of *P. nigra* showed a broad spectrum of antibacterial activity against tested gram negative pathogens. Antibacterial activity of ascidians extracts increased with increasing concentrations. Antibacterial and cytotoxic activity has been previously reported from extracts of some tunicates (Thompson *et al.*, 1985; Ananthan *et al.*, 2011 b; Mohamed Hussain *et al.*, 2011).

The results of the present study showed that highly potent antibacterial substance is found in *P. madrasensis*. Marine ascidians contain antibacterial agent which can be used in relevance to either antifouling technology or clinical pharmacology. Antibacterial activity of the crude extract of ascidian showed inhibitory activity against almost all the four human pathogen strains. The range of inhibiting distance of bacteria varied from 3-12 mm. In some ascidian species, peptides with antibiotic properties *in vitro* have been shown to have other biological effects such as protection against predation, digestion or prevention of surface epibiosis. There is a great scope for finding further novel antimicrobial compounds in the ascidian group, and further research is needed including biochemical and seasonal changes in biologically active peptides. It was concluded from the study that methanolic extract of *P. madrasensis* exhibits potential antibacterial property. Minimum bactericidal concentration was experimented after the MIC in freshly prepared agar plates. After culturing the test organisms separately in nutrient broth containing various concentrations of the active ingredients, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24 h. The lowest concentration of extract that does not yield any colony growth on the solid medium after the incubation period was regarded as minimum bactericidal concentration (MBC) (Alade & Irobi, 1995).

In this study we examined antibacterial activity of the crude methanol and ethanol extract of the test body of *P. madrasensis,* against gram negative strains and it was evident that the gram negative strains were more resistant. On the other hand, Ananthan *et al.* (2011)

reported the maximum antibacterial activity of the crude methanol extracts of the test and mantle bodies of *P. madrasensis* against the isolated urinary tract pathogens *Pseudomonas aerugenosa*. Baquero, (2002) have reported that, some of these bacteria out rightly developed multi drug resistance to some antibiotics. Recently from a 3 year follow up study in USA, Park (2008) reported that, UTI bacterial pathogens have exhibited decreased susceptibility rates to tigecycline over the years. Present results showed moderate antibacterial activity against the multi drug resistant clinical isolates by the test body of *P. madrasensis*. This could be attributed to the fact that the test body might contain secondary metabolites which inhibit the growth of bacteria. This view is consistent with the findings of Paul *et al.,* (2008) reported that the tunicates have the potential to yield novel compounds of ecological, chemical and also biomedical interest. Two new tyrosine derivatives, botryllamides K (1) and L (2), together with six known metabolites, isolated from Australian ascidian, *Aplidium altarium* were evaluated for their cytotoxicity towards the tumor cell lines, MCF-7 (breast), H460 (lung) and SF268 (CNS) (Cardenas, 2001). We conclude that, the continuing and overwhelming contribution of ascidian metabolites to the development of new pharmaceuticals are clearly evident and need to be explored. Antibacterial compounds from natural resources would be the alternative to overcome the resistance problems. The minimum inhibitory concentrations (MICs) and Minimum bactericidal concentration (MBC) of the methanolic extract of *P. madrasensis* are shown in Table 2. The range of MIC varied between 0.72-0.97 mg/ml against all the bacterial strains used in this study and MBC ranges between 0.90-1.20 mg/ml against all the bacterial clinical strains. Ascidians are already reported for rich nitrogenous source with a wide range of biological activities (Biard, 1994).

Table 1: Antibacterial activity of the crude methanol and ethanol extract of *Polyclinum madrasensis* against different gram negative clinical isolates

Zone of inhibition (mm)				
Pathogens	Methanol		Ethanol	
	0.5mg/ml	1mg/ml	0.5mg/ml	1mg/ml
P.aeuroginosa	8	12	6	10
E.coli	4	6	3	5
K.pneumoniae	5	9	5	8
S.aureus	5	7	4	6

Table 2: MIC and MBC methanolic extract of *Polyclinum madrasensis*

Strains	MIC (mg/ml)	MBC (mg/ml)
P.aeuroginosa	0.97	1.20
E.coli	0.72	0.85
K.pneumoniae	0.90	0.95
S.aureus	0.80	0.90

ACKNOWLEDGEMENT

The authors thanks to the authorities of Annamalai University for providing facilities and encouragement.And the Ministry of Earth Sciences (OASTC) New Delhi for Financial assistance.

REFERENCES

1. Alade PI., Irobi ON. Antifungal activities of crude leaf extract of Acalypha wilkesiana. J. Ethnopharmacol.1993, 39:171–174.
2. Ananthan.G., Sivaperumal P., Mohamed Hussain S. Antibacterial Potential of Marine Ascidian *Phallusia arabica* Against Isolated Urinary Tract Infections Bacterial Pathogens. Asian Journal of Animal Sciences. 2011: (5)3 208 – 212.
3. Ananthan G., Mohamed Hussain S., Sivaperumal P., Abdul Jaffar Ali H. Screening For Novel Drug Properties From Marine Tunicate Against Human Urinary Tract Pathogens, Journal of Pharmacy Research.2011a:4(2); 356-357.
4. Ananthan G., Sivaperumal P., Mohamed Hussain S. Cytotoxicity of the crude extracts of Marine ascidians (Tunicata: Ascidiacea) from Tuticorin, Southeast coast of India Archives of Applied Science Research. 2011b, 3 (2):139-142.
5. Azumi K, Yoshimizu M, Suzuki S, Ezura Y and Yokosawa H.Inhibitory effect of halocyamine, an antimicrobial substance from ascidian hemocytes, on the growth of fish viruses and marine bacteria. Cell. Mol. Life Sci. 1990.46(10):1066-1068.
6. Baker JT. Seaweeds in pharmaceutical studies and applications. Hydrobiology.2004, 116/117: 29-40. DOI: 10.1007/BF00027635.
7. Bauer AW., Kirby WM., Sherris JC., Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966: 45(4):493-6.
8. Bergquist PR., The incidence of antibacterial activity in marine demospongiae; Systematic and geographic considerations. Mar Biol.1978.46 (3):215-221.

9. Biard JF., Guyout S., Roassaki C. Lepadi- formine, a new marine cytotoxic alkaloid from Clavelina lepadiformis. Tetrahedran Lett., 1994, 35: 2691-2694.
10. Blunt JW., Copp BR., Hu WP., Munro MHG., Northcote PT., Prinsep MR. Marine natural products. Nat. Prod. Rep, 2007, 24: 31–86.
11. Cardenas F., Thormann M., Feliz M., Caba JM., Lloyd-Williams P., Giralt EJ. Org. Chem. 2001, 66: 4580–84.
12. Carte BK. Biomedical potential of marine natural products. Bio sci.1996.46:271-286.
13. Collins CH., Lyne PM., Grange JM. Microbiological methods. 7th edn. Butterwort – Heinemann Ltd. Britain.1995: pp175–190.
14. Davidson BS. Ascidians: producers of amino acid derived metabolites. Chem. Rev. 1993. 93:1771-1791.
15. Davis AR., Bremner JB. Potential Antifouling Natural Products from Ascidian: A Review. In: Bioactive Compounds from Marine Organisms, Thompson, M.F., R. Sarojini and R. Nagabhushanam (Eds.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 1999. pp: 259-310.
16. Depenbrock H., Peter R., Faircloth GT., Manzanares I., Jimeno J., Hanauske AR. Br. J. Cancer. 1998, 78:739–44.
17. Dowzicky MJ., Park CH. Update on antimicrobial susceptibility rates among gram-negative and gram-positive organisms in the United States: Results from the Tigecycline Evaluation and Surveillance Trial (TEST) 2005 to 2007. Clin. Ther. 2008, 30: 2040-2050.
18. Martinez JL., Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemiology and antibiotic resistance. Clin. Microbiol. Rev. 2002, 15: 647-79.
19. Mohamed HS., Antimicrobial activity of the crude extracts of compound ascidians, *Didemnum candidum* and *Didemnum psammathodes* (Tunicata: Didemnidae) from Mandapam (South East Coast of India), Curr. Res. J. Biol. Sci., 2009.1(3): 168-171.
20. Mohamed Hussain S., Ananthan G., Sivaperumal P. Exploration of cytotoxic potential from marine ascidians *Tridemnum clinides* and *Tridemnum savignii.* Journal of Pharmacy Research. 2011, 4(7): 2032-2033.

21. Moquin-Pattey C., Guyot M. Grossularine-1 and grossularine-2, cytotoxic acarbolines from the tunicate: Dendrodoa grossularia. Tetrahedron. 1989, 45: 3445– 50.
22. Paul VJ., Puglisi MP., Ritson-Williams R. Marine chemical ecology. Nat. Prod. Rep. 2008. 25: 662–695.
23. Proksch P., Edrada RA., Ebel R. Drugs from the sea-current status and microbiological Implications. Appl. Micro. Biotech. 2002, 59:125-134.
24. Rinehart KL., Kobayashi J., Harbpur GC., Hughes RGJ., Miszak SA., Scahill TA. Eudistomins C E K and L, potent antiviral compounds containing a novel oxathiazepine ring from the Caribbean tunicate Eudistoma olivaceum. J. Am. Chem. Soc, 1984, 106: 1524–26.
25. Sato H., Tsuda M., Watanabe K., Kobayashi J. Rhopaladins A–D, New indole alkaloids from marine tunicate Rhopalaea sp. Tetrahedron, 1998, 54: 8687–90.
26. Sivaperumal P., Ananthan G., Mohamed HS. Exploration of antibacterial effects on the crude extract of marine ascidian *Aplidium multiplicatum* against clinical isolates. International Journal of Medicine and Medical Sciences. 2010, 2(12):382-386.
27. Thompson JE., Walker RP., Faulkner DJ. Screening and bioassays for biologically-active substances from forty marine sponge, species from San Diego, California, USA. Mar. Bio. 1985. 88: 11–12.
28. Urdiales JL., Morata P., De Castro IN., Cancer Lett.1996,102:31–37.