

Antimicrobial Activity of the Crude Extracts of Compound Ascidians, *Didemnum candidum* and *Didemnum psammathodes* (Tunicata: Didemnidae) from Mandapam (South East Coast of India)

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Abstract: Many cytotoxic compounds of therapeutic interest have been isolated from marine organisms. In the present study two species of compound ascidians *Didemnum psammathodes* and *Didemnum candidum* were assayed for their antimicrobial activity against eight bacterial and four fungal pathogens. The antimicrobial activity of the crude extract of ascidian shows inhibitory activity against almost all the eight strains. However, *D. psammathodes* shows prominent antibacterial activity than that of *Didemnum candidum*. Whereas no remarkable antifungal activity was noticed against the ascidian crude extract. The maximum inhibition zone (15 mm) was observed against *Salmonella typhi* in the crude methanol extract of *D. psammathodes* and the minimum inhibition zone (1 mm) was noticed against *Vibrio cholera* and *Staphylococcus aureus* in the crude acetone extract of *Didemnum candidum*. In *D. psammathodes* the crude methanol extract the range of inhibition of the bacteria varied from 2–15 mm with an average of 4.3 mm. According to fungi only *Aspergillus niger* and *Penicillium* sp. were showed the trace activity against few crude extract and remaining two fungal pathogens shows negative result. In the present result two way analysis of variance was showing that there was a significant difference between the extracts as well as the strains ($p < 0.05$).

Key words: Antimicrobials, cytotoxicity, marine natural products, *Didemnum* and tunicates

INTRODUCTION

The number of natural products isolated from marine organisms increases rapidly, and now exceeds. With hundreds of new compounds being discovered every year (Faulkner, 2002; Proksch and Muller, 2006). A large proportion of these natural compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and molluscs, and some of them are currently in clinical trials (Proksch, *et al.*, 2002). The need for discovery of new and novel antibiotics is imperative because evidence suggests that development and spreads of resistance to any new antimicrobial agents is inevitable. It has been widely demonstrated that the colonial ascidians are rich in bioactive substances (Wahl, 1995). The case of living marine surfaces the colonization process can additionally be affected by organic metabolites produced by the host organism. These metabolites may affect bacteria in a number of ways, ranging from the induction of a chemo tactic response to the inhibition of bacterial growth or cell death (Bell and Mitchell, 1972; Sieburth and Conover, 1965). The role of secondary metabolites as a chemical defense against epibiosis has been discussed (Bakus, *et al.*, 1986; Davis, *et al.*, 1989; Paul, 1992). The aim of the present study was, first to analyze the antimicrobial activity from the tissue extracts of two colonial ascidians *Didemnum candidum* and *Didemnum psammathodes* were tested against different pathogenic bacterial and fungal strains.

MATERIALS AND METHODS

Collection and preparation of samples: The ascidians *Didemnum candidum* Savigny, 1816 (Chordata: Ascidiacea: Enterogona: Didemnidae) and *Didemnum psammathodes* Sluiter, 1895 (Chordata: Ascidiacea: Enterogona: Didemnidae) were collected during the low tide of the intertidal area at Gulf of Mannar, nearby Central Marine Fisheries Research Institute (CMFRI), Mandapam, Tamil Nadu, (Lat. 9°16' N and Long. 79°8'E), southeast coast of India, between 23 and 25 February 2009. The collected samples were rinsed with sterile sea water to remove associated debris and salt. The samples were weighed (10g) and preserved separately in methanol, ethanol, acetone and chloroform (1:2) and brought to the laboratory. Samples were then soaked in the above mentioned solvents for 48 hrs, the extracts were then obtained from the soaked samples by grinding, using pestle and mortar and filtering through Whatman No.1 filter paper, the filtrate were centrifuged at 3000 rpm. The solvent was evaporated under reduced pressure using desiccators and the residue was weighed and dissolved in the same solvents for using them for the antimicrobial activity. All the Pathogenic bacterial and fungal strains were obtained from Raja Muthiah Medical College, Annamalai University.

Antimicrobial susceptibility assay: Ascidians crude extract was tested for inhibition of bacterial and fungal

growth against human pathogenic bacteria and fungi. Microbial assay were carried out by disc diffusion technique followed by Kelman, *et al.* (2001). All the bacterial strains were enriched in nutrient broth at 37°C for 18-24 hours, after which they were streaked over Mueller Hinton agar surface using sterile cotton swabs. *In vitro* antifungal activity of ascidian crude extract was determined against in Czapek Dox agar, inoculums of 24 hour old culture for *Aspergillus niger*, *A. flavus* and *A. fumigates* well drained spores were distributed uniformly on the surface of the agar plates with the help of sterile cotton swab. Other fungal strains were inoculated by taking a piece of fungal colony using a sterile cotton swab and gently swabbed on the surface of the medium. Then 20 µl of the extract was pipetted on a 6 mm sterile paper disc, the solvent was allowed to evaporate and the disc was placed on the surface of the plate. The plates were incubated for 24 h at 37°C. Areas of inhibited bacterial and fungal growth were observed as clear halos (zones) around the disc. Antimicrobial activity was measured as diameter of zone of inhibition, excluding the paper disc diameter. Inhibition zone was observed after 72 h.

RESULTS AND DISCUSSION

Totally eight crude extracts from the two species of ascidians were screened against eight human pathogenic bacteria and four fungal pathogens for testing their antimicrobial activities. The inhibition zones of extract against the specific test organisms were given in Fig. 1 and 2. The maximum inhibition zone (15 mm) was observed against *S. typhi* in the crude methanol extract of *D. psammathodes* and the minimum inhibition zone (1 mm) was noticed against *Vibrio cholera* and *Staphylococcus aureas*. According to fungi only *Aspergillus niger* and *Penicillium* sp. were showed the trace activity against few crude extract and remaining two fungal pathogens showed negative results. In the present study crude methanol extract of *D. psammathodes*, the range of inhibition of the bacteria varied from 2-15 mm with an average of 4.3 mm, in ethanol extract, the range varied from 3-8 mm with an average of 3.62 mm, in acetone extract the range varied from 2-3.5 mm with an average of 1.81 mm and chloroform extract the range varied from 2-3 mm with an average of 0.93 mm. Santhana Ramasamy and Murugan (2003) has reported that the crude methanol extract of *D. psammathodes*, the range of inhibition of the bacteria varied from 6 and 10 mm with an average of 7.1 mm. In the present study *D. candidum* the crude methanol extract the range varied from 1-10 mm with an average of 2.12 mm, in ethanol extract varied from 2-5 mm with an average of 1.75 mm, in acetone extract the range of inhibition was 1 mm with an average of 0.5 mm and chloroform extract the range varied from 1-2 mm with an average of 0.37 mm. The crude extract of *D. psammathodes* exhibited strong antibacterial activity of almost all the strains tested in this

study, comparatively, *D. candidum* exhibited low inhibitory activity. Prem Anand and Patterson Edward (2002) reported that comparatively the ascidians *D. psammathodes* seems to be a promising source of antibacterial compound. The crude methanol extract of *D. psammathodes* shows the maximum antibacterial activity against *Salmonella typhi*, followed by *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio cholera* and the minimum activity was noticed against *Proteus mirabilis* and there was no activity in *Staphylococcus aureas*. The same crude extract of *D. candidum* exhibited the maximum antibacterial activity against *S. typhi*, followed by *B. subtilis*, the minimum was recorded in *P. mirabilis* remaining five strains fails to shows the antibacterial activity against the crude extract of *D. candidum*. In crude ethanol extract of *D. psammathodes* shows maximum activity against *S. typhi* followed by *P. mirabilis*, *S. aureas*, *B. subtilis*, *V. cholera* and the minimum was noticed against *E. coli*, no activity was found against *P. vulgaris* and *P. aeruginosa*. In crude ethanol extract of *D. candidum* shows maximum activity against *S. typhi*, followed by *S. aureas*, *B. subtilis*, *E. coli* and the minimum activity was noticed against *V. cholera*, whereas the remaining three pathogens fails to showed the antibacterial activity. The crude acetone extract of *D. psammathodes* shows the activity against only four of the eight strains, maximum activity was noticed against *P. mirabilis* followed by *S. typhi*, *S. aureas* and minimum was recorded against *V. cholera*. Prem Anand and Patterson Edward (2002) reported that in *D. psammathodes* the highest activity was seen against *P. mirabilis* (7 mm), *Shigella flexneri* (8 mm) and *Salmonella typhi* (6 mm). In the present study acetone extract of *D. candidum* shows the activity against only three of the eight strains, maximum was noticed against *B. subtilis*, followed by *P. mirabilis* and minimum was noticed against *S. aureas*. In chloroform extract of *D. psammathodes* represent the activity against only four of the eight strains maximum was noticed against *P. vulgaris*, followed by *S. aureas*, *P. mirabilis* and minimum was noticed against *V. cholera*. In chloroform extract of *D. candidum* shows the activity against only four of the eight strains, the maximum was recorded in *B. subtilis* followed by *P. mirabilis*, *S. typhi* and the minimum was recorded against *V. cholera*. In the present study maximum inhibition zone (15 mm) was observed in the crude methanol extract and (8 mm) inhibition zone was noticed in the crude ethanol extract of *D. psammathodes* and the minimum inhibition zone (1 mm) was recorded against *Vibrio cholera* and *Staphylococcus aureas* in the crude acetone extract of *D. candidum*. The results of present study similar to that the previous report, Murugan and Santhana Ramasamy (2003) reported that the methonal water extract of *Distaplia nathensis* exhibited antimicrobial activity against 12 microbes of the 14 species studied, no effect was observed on *Pseudomonas* sp. and *Vibrio* sp. The present study *D. psammathodes* consist to be a promising source of antibacterial activity Biard, *et al.* (1994) observed that ascidians are already reported to be a rich

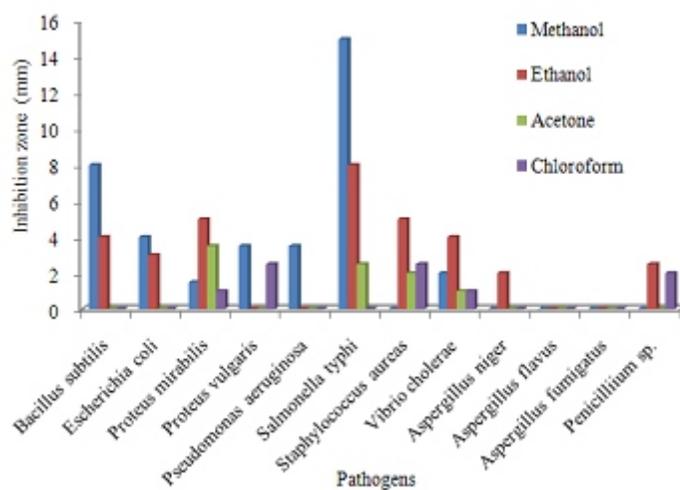


Fig. 1: Antimicrobial activity of *Didemnum psammathodes*

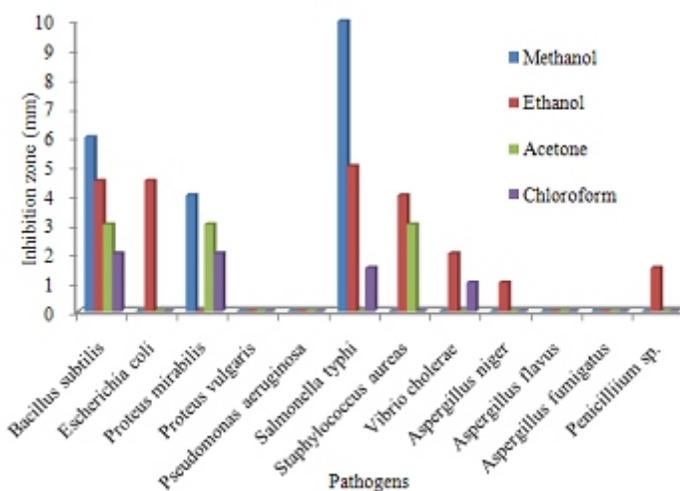


Fig. 2: Antimicrobial activity of *Didemnum candidum*

source of nitrogen compounds with a wide range of biological activities. In the present study there was no antifungal activity against the crude methanol extract of both *D. psammathodes* and *D. candidum*. In ethanol extract of *D. psammathodes* shows the activity of (2 mm) against *Aspergillus niger* (2.5 mm) against *Penicillium* sp. *D. candidum* shows the activity of (1.5 mm) against *Penicillium* sp. and (1 mm) against *A. niger*. whereas no activity was observed in the remaining two strains. Prem Anand and Patterson Edward, (2002) was observed the crude methanol extract of *D. psammathodes* exhibited wide spectral antibacterial activity but no anti fungal activity. In the present study also no anti fungal activity was observed in acetone extract of both the ascidian samples. In chloroform extract *D. psammathodes* were showed activity of (2 mm) and *D. candidum* shows (1 mm) against *Penicillium* sp. whereas no anti fungal activity

was observed in rest of the three fungal pathogens against chloroform extract Ronald, (1997) has reported that the fungi are more resistant than the bacterial strains to the tested compound, this could be leads to the nature of fungal cell wall made up of chitin, the hard cover of the exoskeletons of the arthropods are also made up of chitin, which is relatively resistant, including microbial decomposition. In the present study *D. psammathodes* the crude ethanol extract the range of inhibition of the fungi varied from 2-2.5 mm with an average of 1.12 mm, in chloroform extract the range of inhibition of the fungi was 2mm with an average of 0.5 mm. In ethanol extract of *D. candidum* the range varied from 1-1.5 mm with an average of 0.62 mm was noticed. In the present result two way analysis of variance was showing that there was a significant difference between the extracts as well as the strains ($p < 0.05$) was given in (Table 1).

Table 1: Two way ANOVA for antimicrobial activity

Source of Variation	SS	df	MS	F	P-value	F crit
Between extracts	59.1875	3	19.72917	3.49916	0.026173	2.891564
Between strains	132.7292	11	12.06629	2.140074	0.045157	2.093254
Error	186.0625	33	5.638258			
Total	377.9792	47				

In recent years, great attention has been paid to study the bioactivity of natural products, because of their potential pharmacological utilization. Most homeopathic medicines are either from plant or animal origin was reported by Ray and Mukherjee, (1979). The first attempt to locate antimicrobial activity in the marine organism was initiated around 1950's has reported by Berkholder and Berkholder, (1958). Organic substances produced by marine plants and animals have been shown to affect bacterial behavior was reported by Bell and Mitchell, (1972). The extent to which secondary metabolites function as an antibacterial chemical defense however, has not been demonstrated (Bakus, *et al.*, 1986; Paul, 1992). The most potent metabolite from ascidian discovered is Didemin-B from a Caribbean tunicate *Trididemnum solidum*, the first marine compound in human cancer clinical trails as a purified natural product was reported by Davidson, (1993).

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REFERENCES

Bakus, G.J., N.M. Targett and B. Schulte, 1986. Chemical ecology of marine organisms: An overview. *J. Chem. Ecol.*, 12: 951-987.

Bell, W. and R. Mitchell, 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biol. Bull.* 143: 265-277.

Berkholder, P.R. and L.M. Berkholder, 1958. Antimicrobial activity of horny corals. *Science*, 127: 174.

Biard, J.F., S. Guyot C. Roassaki and J.F. Verbist, 1994. Lepadiformine, a new marine cytotoxic alkaloid from *Clavelina lepadiformis*. *Tetrahedron Lett.*, 35: 2691- 2694.

Davidson, B.S., 1993. Ascidiates: Procedures of amino acid derived metabolites. *Chem. Rev.*, 93: 1771-1791.

Davis, A.R., N.M. Targett, O.J. McConnell and C.M. Young, 1989. Epibiosis of Marine Algae and Benthic Invertebrates: Natural Products Chemistry and other Mechanisms Inhibiting Settlement and Overgrowth. In: *Bioorganic Marine Chemistry*. P.J. Scheuer, (Ed.). Springer-Verlag, Berlin, pp: 85-114.

Faulkner, D.J., 2002. Marine natural products. *Nat. Prod. Rep.*, 19: 1-48.

Kelman, D., Y. Kashman, E. Roserberg, M. Ilan, I. Ifrach and Y. Loya, 2001. Antimicrobial activity of reef sponge *Amphimedon viridis* from the Red sea: evidence for selective toxicity. *Aquat. Microb. Ecol.*, 24: 9-16.

Murugan, A. and M. Santhana Ramasamy, 2003. Biofouling deterrent activity of the natural product from ascidian, *Distaplia nathensis* (chordata). *Indian. J. Mar. Sci.*, 3: 162-164.

Paul, V. J., 1992. Chemical Defenses of Benthic Marine Invertebrates. In: *Ecological Roles of Marine Natural Products*. V.J. Paul, (Ed.). Cornstock Publ. Ass., Ithaca, pp: 164-188.

Prem Anand, T. and J. K. Patterson Edward, 2002. Antibacterial activity in the tissue extracts of five species of *Cowries cypraea* spp. (Mollusca : Gastropoda) and ascidian *Didemnum psammathodes* (Tunicata : Didemnidae) *Indian. J. Mar. Sci.*, 31: 239-242.

Proksch, P., R.A. Edrada, R. Ebel, 2002. Drugs from the sea-current status and microbiological implications. *Appl. Microbiol. Biotechnol.*, 59: 125-134.

Proksch, P., and W.E.G. Muller, 2006. *Frontiers in Marine Biotechnology*. Horizon Bioscience: Norfolk, U.K.

Ray, P.G. and S.K. Mukherjee, 1979. Screening of homeopathic mother tinctures for antimicrobial activity. *Indian J. Exp. Biol.*, 15: 338-339.

Ronald, M.A., 1997. *Principles of Microbiology*, C. Brown Publishers, pp:1298.

Sieburth, J.M. and J. T. Conover, 1965. *Sargassum tannin*, an antibiotic which retards fouling. *Nature*, 208: 52-53.

Wahl, M., 1995. Bacterial epibiosis on Bahamian and Pacific ascidiates. *J. Exp. Mar. Biol. Ecol.*, 191: 239-255.