

Antibacterial Activity of Methanol Extract of Colonial Ascidian *Distaplia nathensis*

¹V. Chandramathi, ²R.D. Thilaga and ³V. Sivakumar

¹Spic Research Centre, V.O. Chidambaram College, Thoothukudi-628008, TamilNadu, India

²Department of Zoology, St. Mary's College, Thoothukudi-628008, TamilNadu, India

³Department of Zoology, V.O. Chidambaram College, Thoothukudi-628008, TamilNadu, India

Abstract: Ascidiaceans have some pronounced pharmacological bioactive active compounds which are useful in biomedical arena. Methanol and benzene fractionated extracts of ascidian, *Distaplia nathensis* were assayed for antibacterial activity against 6 human pathogens. All the fractions showed inhibitory activity against the majority of the tested strains. Of the five fractions, the methanol extract (A₅) developed maximum inhibition zones against all pathogens. Thus the non toxic extract of ascidian is a genuine source of antibacterial drugs for human use but it needs further investigations.

Key words: Ascidian • Antibacterial • Bioactive compounds

INTRODUCTION

Ascidiaceans are sessile marine filter feeders in the phylum chordate that are common in benthic marine environments [1]. Tunicates have been reported to be rich sources of biologically active compounds and ranked the third for their overall activities, next to sponges and bryozoans [2]. The tunicate (ascidiaceans) *Trididemnum solidum* is the first marine compound to enter human cancer clinical trial as a purified natural product [3]. This class of cyclic peptide provides important structural lead for a variety of cytotoxic, antiviral, anticancer and immunosuppressant activities [4, 5]. The present study was carried out to analyze the antimicrobial activity of the tissue extracts of colonial ascidian *Distaplia nathensis* on different bacterial strains.

MATERIALS AND METHODS

The Ascidiaceans *Distaplia nathensis* (Fig. 1a) were collected during the low tide from the inter tidal area of Gulf of Mannar, Thoothukudi (lat.8° 45'N; long 78° 10'E) Tamil Nadu, South east coast of India. The collected samples were rinsed with sterile sea water to remove associated salts and debris. The animals were dried in a hot air oven at 40°C for 24 hrs and powdered. 25gms of the powder was soaked in methanol (150ml) and the crude extract was obtained [12] and fractionated using different solvent system viz. Benzene (200ml), Benzene: Methanol

3:1, Benzene: Methanol 2:2, Benzene: Methanol 1:3, Methanol (200ml) by column chromatography and was considered as A₁, A₂, A₃, A₄ and A₅. The solvent was evaporated and the residue was weighed and dissolved in the same solvents for the antimicrobial activity.

The crude extract of the ascidian was tested for inhibition of bacterial growth against human pathogenic bacteria. Microbial assay was carried out by disc diffusion technique [6]. All the bacterial strains were enriched in nutrient broth at 37°C for 18-24 hrs, after which they were streaked over nutrient agar (Himedia, Mumbai, India) surface using sterile cotton swabs. Then 200 µl of the extract was pipetted on a 6mm 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 hrs at 37°C Antibacterial activity was measured as the diameter of zone of growth inhibition, excluding the paper disc diameter. The inhibition zone was observed and measured after 72 hrs.

RESULTS AND DISCUSSION

The crude methanol extract of the ascidian *D. nathensis* was tested against six human pathogens. The inhibition zones of extract against the specific test organisms were given in (Fig. 1b). Of the five fractions obtained, fraction five (A₅) developed maximum inhibitory zones against all pathogens viz. *Salmonella typhi* (20mm), *Klebsiella pneumoniae* (18mm), *Escherichia coli* (17mm),



Fig. 1a

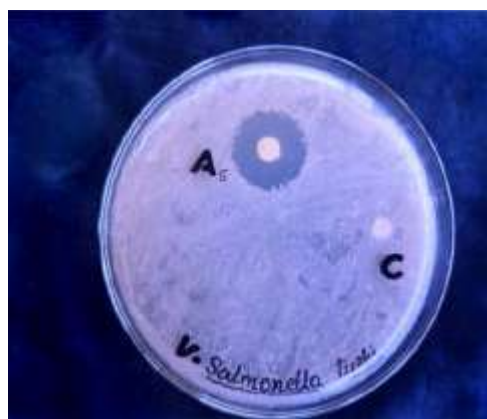


Fig. 1b

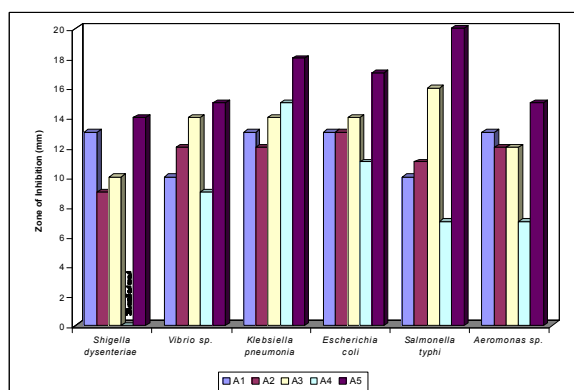


Fig. 1c: Antibacterial activity of *Distaplia nathensis* in different fractions

Vibrio sp. (15mm), *Aeromonas sp.* (15mm) and *Shigella dysenteriae* (14mm) (Fig. 1c). The range of inhibition of the bacteria varied from 10-13mm in Benzene extract (A₁), 9-13mm in Benzene: Methanol (3:1) (A₂) extract, 10-16mm in Benzene: methanol (2:2) (A₃) extract, 7-15mm in Benzene: Methanol (1:3) (A₄) extract. A₄ showed no activity against *Shigella dysenteriae*. Crude methanol

extract of *Didemnum psammathodes* showed range of inhibition of bacteria varied from 6 and 10mm with an average of 7.1mm [7]. The crude methanol extract of *Didemnum candidum* show inhibition zone varied from 1-10mm with an average of 2.12mm, that of ethanol extract varied from 2 - 5mm with an average of 1.75mm, (Fig. 1a) acetone extract showed the range of inhibition 1mm with an average of 0.5mm and with chloroform extract the range varied from 1-2mm with an average of 0.37mm [8]. Crude methanol extract of *Phallusia nigra* exhibited maximum antibacterial activity against *Staphylococcus aureus* [9].

Antibacterial activity of *Polyclinum madrasensis*, *Herdmania pallida* and *Didemnum psammathode* against 10 different bacterial pathogens were studied by many scientists [8, 10, 11]. Crude methanol extract of all the above ascidian species showed the highest activity against the pathogens. With *Didemnum psammathodes* the highest activity was seen against *Salmonella typhi* (6mm) *Proteus mirabilis* (7mm), *Shigella flexneri* (8mm) [12].

In general, ascidians are known to contain a variety of novel and highly bioactive compounds which have been hypothesized to function in chemical defense [9]. *Distaplia nathensis* different fractions can be considered a promising source of antibacterial activity. However, further investigations involving application of the extracts as drugs for human administration are needed.

ACKNOWLEDGEMENT

The authors express their immense gratitude to R.D. Thilaga, Associate Professor, Dept. of Advanced Zoology and Biotechnology, St Mary's College, Thoothukudi and Dr. V. Sivakumar, Associate professor, P.G. Research Dept of Zoology, V.O.C. College, Thoothukudi for their support and encouragement.

REFERENCES

1. Swalla, B.J., C.B. Cameron, L.S. Corle and J.R. Garey, 2000. Urochordates are monophyletic within the deuterostomes. *Systematic Biol.*, 49: 52-64.
2. Davis, AR. and J.B. Bremner, 1999. Potential antifouling natural product from ascidian: In: *Bioactive compounds from marine organisms*. Eds. M.F. Thompson, R. Sarojini and R. Nagabhushanam. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi, pp: 259-310.
3. Carte, B.K., 1996. Biomedical potential of marine natural products. *Bioscience*, 46: 271-286.

4. Sakai, R., J.C. Stroch, D.W. Sullins and K.I. Rinehart, 1995. Seven new didemnins from the marine Tunicate *Trididemnin solidum*. J.Am. Chem: Soc., 117: 3734-3748.
5. Manuel Martinez-Gercia, Marta Diaz-Valdes, Alfonso Ramos-Espla, Nelida Salvador, Patricia Lopez, Eduardo Larriba and Josefa Anton, 2007. Cytotoxicity of the Ascidian *Cystodytes dellechiajei* Against Tumor cells and Study of the Involvement of Associated Microbiota in the production of cytotoxic Compounds. Mar. Drugs, 5: 52-70.
6. Kelman, D., Y. Kashman, E. Rosenberg, 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.
7. Santhana Ramasamy, M. and A. Murugan, 2003. Chemical defence in ascidians *Eudistoma viride* and *Didemnum psammathodes* in Tuticorin, Southeast coast of Indian. Indian J. Mar. Sci., 32(4): 337-339.
8. Mohamed Hussain and Ananthan, 2009. Antimicrobial Activity of the crude Extracts of compound Ascidians, *Didemnum candidum* and *Didemnum psammathodes* (Tunicata: Didemnidae) from Mandapam (South East coast of India). Current Research J. Biological Sci., 1(3): 168-171.
9. Abdul Jaffer Ali, H., M. Tamil Selvi and V. Sivakumar, 2008. Antibacterial activity of the marine ascidians *Phallusia nigra* and *Herdmania pallida* from the Tuticorin coast, India. J. Biological Research Thessaloniki, 10: 171-179.
10. Pawlik, J.R., 1993: Marine invertebrate chemical defenses. chem. Rev., 98: 1911-1922.
11. Karthikeyan, M.M., G. Ananthan and T. Balasubramanian, 2009. Antimicrobial Activity of crude Extracts of some Ascidians (Urochordata: Ascidiacea) from Palk strait. (South coast of India). World J. Fish and Marine Sci., 1(4): 262-267.
12. 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.