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# ASSESSMENT OF ANTIMICROBIAL POTENTIAL OF THE ASCIDIAN *DIDEMNUM PSAMMATODE*, FROM ANDAMAN SEA

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## ABSTRACT

*Ascidians are crucial group for the evolutionary studies of deuterstomes and origin of chordates. Most of the literature has laid emphasis on its diversity, species composition and evolutionary history and not much has been highlighted on its antimicrobial potential. In the present study, antibacterial activity of the ascidian, Didemnum psammatode tested against human clinical pathogens by disc diffusion method is being reported. Crude methanol extract (50 µl) exhibited maximum inhibition zone (22 mm) against Shigella sonnei at 40 mg/ml concentration while the minimum inhibition zone of 12.5 mm was observed against E.coli. The results obtained shows antibacterial potential of crude extract of Didemnum psammatode against the human pathogenic strains.*

## KEY WORDS

*Ascidians, Didemnum psammatode, Antimicrobial activity, Disc diffusion method*

## INTRODUCTION

The marine environment is endowed with great dimensions of uncharted and unexplored realms, unimaginable and non-imbibable, as a whole, for trivial beings like humans. One of the dubious amazement is the evolution of life from one form to another. A vast biotic community exists, intersecting boundaries of various spheres of living species, indicating variably immense and robust composition, diversity and model structures of species. The massive pool of marine biodiversity are excellent natural reservoirs for indenting an directory of bioactive molecules with promising potential for biotechnological applications (McBee 1971; Hungate 1975).

Ascidiacea, a class of lower metazoans, classified under phylum Chordata and subphylum Urochordata (or Tunicata), are considered to be an evolutionary connecting link between invertebrates and vertebrates. The term "Tunicata" hails from the polysaccharide (tunicin) containing tunic that envelopes the animal and forms a flexible skeleton (Monniot *et al*., 1991) that includes both, sessile and planktonic species, characterized by the presence of a 'tunic' composed of complex polysaccharides.

Ascidians comprises of about 3000 species inhabiting shallow waters, spanning from tropical to polar waters. Around, 359 species (10 families and 38 genera) have been reported from India (Meenakshi, 2010) and 32 species have been documented from Andaman group of Islands. *Didemnum*, *Phallusia*, *Polycarpa*, *Herdmania* and *Pyura* are some of the most abundant genera found along the Andaman coast (Mondal *et al*., 2015).

Ascidians, one of the major groups, have been increasingly reported as bioinvasive species, affecting largely local ecological communities (Castilla *et al*., 2014) and as macrobiofoulers (Deepa *et al*., 2015) resulting in huge economic and monetary losses. Despite, their bioinvasive nature, they have been gaining popularity, due to their bioactive novel chemicals, found to have a broad spectrum of anti-

microbials and ranks next to sponges and bryozoans, for their overall activities (Davis & Bremner, 1999). These compounds comprise of various derivatives of alkaloids and peptides, involved in chemical weaponry system, which helps in evading the pathogens, due to specialized cells like vanadocytes in their test (Hawkins *et al*., 1983).

Methanolic and ethanolic extracts from ascidians have found to be biologically active against clinical pathogens like *Vibrio cholera*, *Aspergillus niger*, *Shigella boydii* and *Salmonella paratyphi* (Sluiter, 1895). Ascidians from genus *Didemnum* presently are being used to produce anti-cancer drugs (Karthikeyan *et al*., 2009). *Botryllus* sp. and *Didemnum* sp. have been reported for producing anti-cancer drugs (Azumi *et al*., 1990). Azumi *et al*., (1990) isolated Halocyamine A, an antimicrobial substance from haemocytes of the solitary ascidian, *Halocynthia roretzi*.

The present work focuses on antimicrobial property of *Didemnum psammatode* isolated from Burmanallah coast against pathogenic bacterial strains.

## MATERIALS AND METHODS

### Sampling station

Burmanallah (11° 34.505’ N, 92° 44.275’E) was selected for the study owing to its wide intertidal region, which spans up to 220 m during lowest low tide (Fig.1). Samples (*Didemnum psammatode*) (Fig. 2) were collected by using hand picking method through Line intercept transect and photoquadrat method (English *et al*., 1997), transferred into sterile vials and kept at 4°C until further analysis.


**Fig. 1: Map showing study area Burmanallah (11° 34.505’ N, 92° 44.275’E)**

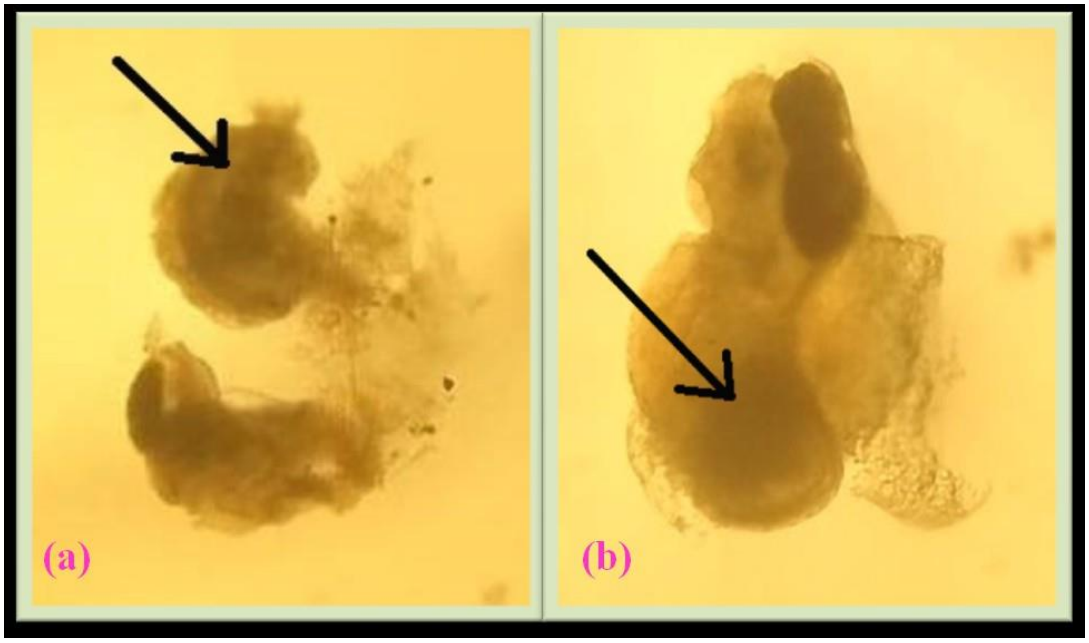


**Fig. 2: *Didemnum psammatode* (Sluiter, 1895): (a) whole zooid (arrow: with four distinct rows of stigmata); (b) abdomen of zooid (arrow: with tightly coiled vas deferens).**

**Identification of samples**

For identification, the fixed specimens were dissected under a stereomicroscope (Nikon SMZ1500) using sterile fine blade, needles and forceps and were identified by comparing aforesaid intricate details like structure of vas deferens, number of rows of stigmata, shape of the gut, nature of gonads etc., by following the techniques of Kott (1985, 2001). Furthermore, specimens of species containing spicules were treated with 3% HCl for decalcification before dissection and further processing which makes it easier to remove zooids fixed in the test and minimizes the physical damage to keep their bodies intact.

**Culture maintenance and storage**

Ten pathogenic bacterial strains (*Shigella flexneri* 503004, *S. sonnei* NK4010, *S. dysenteriae* Type 5, *Vibrio fluvialis* IDH2036, *V. cholera* Co835, ETEC 0115, STEC 0517: H7, *Salmonella typhi* B12101, *S. typhi* C6953, *Aeromonas hydrophila* IDH1585), maintained in our laboratory, were used, cultured and maintained in nutrient agar following standard aseptic microbiological techniques. For inoculum preparation, bacterial cultures were inoculated in sterile nutrient broth tubes and incubated at 37°C for 20-24 h.

**Preparation of Methanolic Extract**

The collected sample was cut into small pieces using sterile scissors and dried overnight in a hot air oven at 57°C. After drying, the sample was weighed and homogenized using mortar and pestle in sterile conditions. The sample was then extracted with methanol at room temperature for 48 hr, filtered through Whatman No.1 filter paper and concentrated using rotary evaporator (Buchi) at 30°C under vacuum (Melba *et al*., 2013). The crude methanolic extract was then diluted with methanol to a final concentration of 40 mg/ml and analysed for its antibacterial properties using standard disc diffusion method.

**Antibacterial assay**

Antibacterial activity of *Didemnum psammatode* crude extract was assessed by disc diffusion method (Bauer and Kirby, 1966). Inoculum (0.1 ml) were plated on Mueller Hinton agar plates and the discs impregnated with 50 µl crude extract (40 mg/ml), methanol (negative control) and Gentamicin (10 mcg) (positive control) were placed at uniform distances from each other incubated at 37°C for 24 hr. Growth inhibition zones produced were measured and compared to those of the controls.

## RESULTS AND DISCUSSION

In-vitro antibacterial screening of methanolic extract of ascidian, *Didemnum psammatode* against tested pathogenic strains were performed and the zone of inhibition were measured (Fig. 3 and 4). Maximum inhibition zone of 22 mm was observed against *Shigella sonnei* followed by *Salmonella typhi* B12101 and C6953

type strains (17 mm each), *Vibrio cholera* (15 mm), *Shigella dysenteriae* Type 5 and Enterotoxicogenic *E.coli* (14.5 mm each) and *Shigella flexneri* (14 mm). The results correspond with the findings of Mohamed *et al*. (2009). Similar study has also been reported by Selva *et al*. (2011) in case of crude methanol extract of *Phallusia arabica* which shows the variable inhibitory zones of 4-12 mm with an average of 7 mm. Minimum activity was observed in STEC 0517: H7 (12.5 mm), *Aeromonas hydrophila* (13 mm) and *Vibrio fluvialis* (13.5 mm). No inhibition zone was observed in methanol (50 µl).

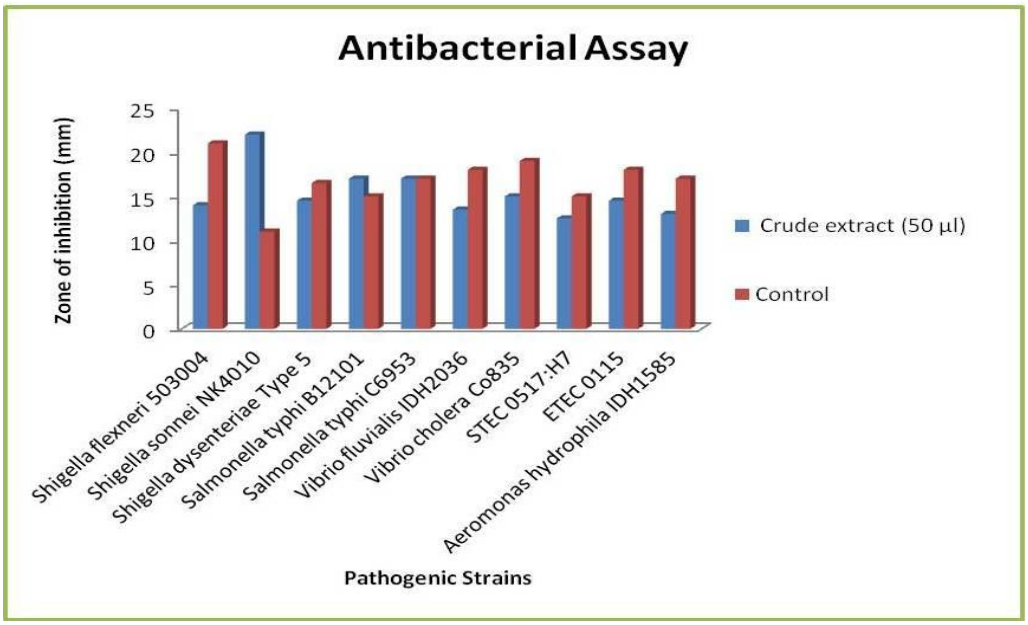


**Fig. 3: Antibacterial activity of methanolic extract of *Didemnum psammatode* against tested human pathogens.**

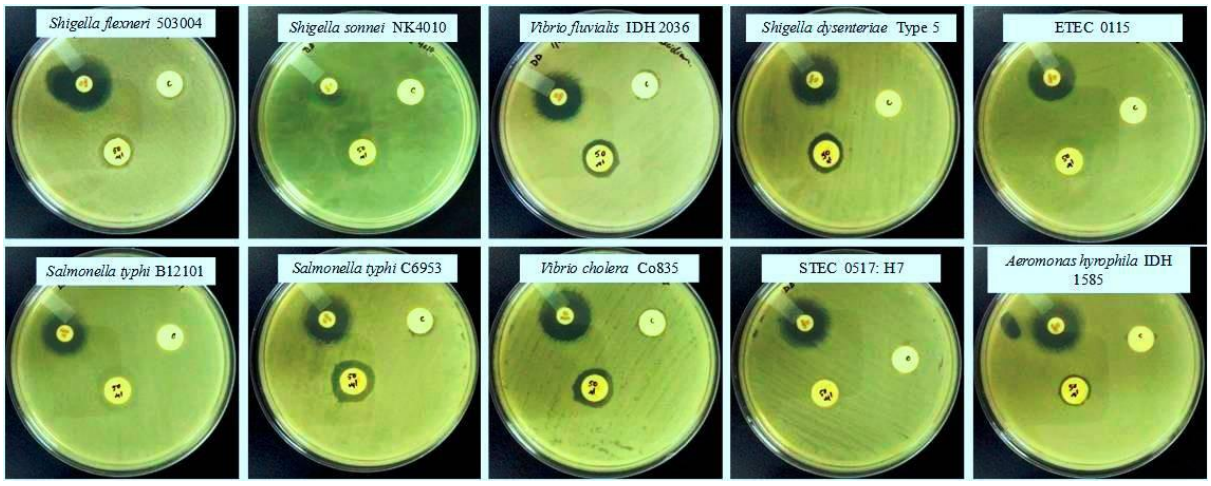


**Fig. 4: Growth Inhibition Zones produced by crude extract of *D. psammatode* against tested pathogenic strains**

Cameron *et al*. (2004) reported the potential antibacterial activity of methanolic extracts of ascidians. Significant antibacterial activity against *E. coli*, *Agrobacterium tumifaciens*, *S. aureus* and *P. aeruginosa* from the extracts of *Cynthia savignyi*, a Morocco Atlantic sea ascidian, was reported by Abourriche *et al*. (2003).

Many scientific reviews have exemplified that ascidians possess potential novel compounds of chemical, ecological and biomedical interest (Paul *et al*., 2008). *Tridemnum*, a cosmopolitan genus, is well renowned for its variability in chemical metabolites for producing first marine compound, Tridemnum solidum, as human cancer drug which enlists cyclic peptides, providing a vital structural lead for a variety of cytotoxic, antiviral, immunosuppressant and anticancer activities (Sakai *et al*., 1995; Carte, 1996).

However, a number of bioactive natural compounds has been isolated from tunicates and not much, about their ecological roles and distributional patterns within the ascidian body tissues, are known (Pisut *et al.*, 2002; Avila *et al.*, 2008). Marine genus synthesizes active constituents which can be used in traditional and complementary medicines, thus, offering a novel route of antibacterial compounds from natural sources, alternative to overcome the drug resistant phenomena of clinical pathogens (Anuba *et al*., 2009). Thus, the ascidian, *Didemnum psammatode* seems to be a promising source of antibacterial compounds which could be used in pharmacological research. Further studies are needed for the purification and structural elucidation of antibacterial drugs and secondary metabolites.

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**REFERENCES**

Abourriche A., Abboud Y., Maoufoud S., Seffaj H., Charrout M., Chaib N., Bennamara A., Bontemps N., Francisco C., Cynthichlorine: A bioactive alkaloid from the tunicate *Cynthia savignyi*. II framaco, 58: 1351-1354, (2003)

Anbu-Jeba-Sunilson J., Suraj R., Anandaraja gopal K., Rejitha G., Vignesh M. and Promwichit P., Preliminary phytochemical analysis, elemental determination and antibacterial screening of *Codium decorticatum*-a marine green algae. Int. J. Biol. Chem., 3: 84-93, (2009)

Avila C., Taboada S., Nunez-Pons L., Marine Antarctic chemical ecology: What is next? Mar. Ecol., 29: 1–70, (2008)

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., 46 (10): 1066-1068, (1990)

Bauer A.W., Kirby W.M.M., Sherris J.S., and Turk M., Antibiotic susceptibility by standardized single disk method. American J. Clinical Patho., 45: 493-496, (1966)

Cameron F., Valerie J.S., Antibacterial activity in the blood cells of the solitary ascidian, *Ciona intestinalis*, in vitro. Journal of Experimental Zoology, 273 (5): 434 – 444, (2004)

Carte B.K., Biomedical potential of marine natural products. Bio sci, 46: 271-286, (1996)

Castilla J.C., Manriquez P.H., Delgado A., Ortiz V., Jara M.E., Varas M., Rocky intertidal zonation pattern in Antofagasta, Chile: Invasive species and shellfish gathering. PLoS ONE, 9 (10); e110301, (2014)

Davidson B.S., Ascidians: Producers of amino acid derived metabolites. Chem. Rev., 93: 1771-1791, (1993)

Davis, A.R., Bremner, J.B., in: Thompson, M.F., Sarojini, R., Nagabhushanam, R. (Eds.), Bioactive compounds from marine organisms, Oxford and IBH Publishing Co., New Delhi, pp. 259-310, (1999)

Deepa S., Sathish T., Vinithkumar N. V., Limna Mol V. P., Kirubagaran R., Distribution and diversity of macrofoulers in the Coastal areas of Port Blair, Andaman and Nicobar Islands. Int. J. Environ. Res., 9 (4): 1315-1324, (2015)

English, S., Wilkinson, C., Baker, V., Manual for survey of tropical marine resources. 34-51, (1997)

Hawkins C.J., James G.A., Parry D.L., Swinehart J.H. and Wood A.L., Intracellular acidity in the ascidian. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 76 (3): 559-565, (1983)

Hungate R.E., The rumen microbial ecosystem. Annu. Rev. Ecol. Evol. Syst., 6: 39–66, (1975)

Karthikeyan M.M., Ananthan G., Balasubramanian T., Antimicrobial activity of crude extracts of some ascidians (Urochordata: Ascidiacea) from Palk Strait (Southeast Coast of India). World Journal of Fish and Marine Sciences, 1 (4): 262-267, (2009)

Kott P., The Australian Ascidiacea, Part 1 Phlebobranchia and Stolidobranchia. Memoirs of the Queensland Museum, 23: 1-440, (1985)

Kott P., The Australian Ascidiacea, Part 4 Aplousobranchia (3), Didemnidae. Memoirs of the Queensland Museum. 47: 1-408, (2001)

McBee H., Significance of intestinal microflora in herbivory. Annu. Rev. Ecol. Evol. Syst., 2: 165–176, (1971)

Meenakshi V.K., Indian ascidians- Potential candidate for research- A Review. Indian Journal of Biotechnology, 1: 29-33, (2010)

Melba C.C., Ananthan G., Stalin C., Antibacterial activity of the crude extract of *Trididemnum savignii* (Herdman 1886) against clinical pathogens. International Journal of Pharmaceutical, 2 (4): 1-5, (2013)

Mohamed Hussain S., Ananthan G., 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., 1 (3): 168-171, (2009)

Mondal J., Raghunathan C., Mondal T., Diversity and distribution of common ascidians of Andaman group of islands. Middle-East Journal of Scientific Research, 23 (10): 2411-2417, (2015)

Monniot C., Monniot F., Laboute P., Coral Reef Ascidians of New Caledonia, Collection Fauna Tropicale 30, ORSTOM, Paris (1991)

Paul V.J., Puglisi M.P., Ritson-Williams R., Marine chemical ecology. Nat. Prod. Rep., 25: 662–695, (2008)

Pisut D.P., Pawlik J.R., Anti-predatory chemical defenses of ascidians: Secondary metabolites or inorganic acids? J. Exp. Mar. Biol. Ecol., 270: 203–214, (2002)

Sakai R., Stroch J.C., Sullins D.W., Rinehart K.I., Abdul Jaffer Ali H., Tamil Selvi M. and Sivakumar V., Antibacterial activity of the marine ascidians Tunicate *Tridemnin solidum, Phallusia nigra* and *Herdmania pallida*. J.Am. Chem: Soc.,117: 3734-3748, (1995)

Selva Prabhu A., Ananthan G., Antibacterial activity of ascidian *Phallusia arabica* against human clinical isolates. Journal of Applied Pharmaceutical Science, 01 (10): 143-145, (2011)

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