INVESTIGATION OF ANTIBACTERIAL POTENTIAL OF ASCIDIAN, MICROCOSMUS EXASPERATUS (Heller, 1878) AGAINST HUMAN URINARY TRACT PATHOGENS

*G. Ananthan and K. Iyappan
Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai- 608 502

ABSTRACT
The current investigation is based on the discovery of novel antibacterial from new sources which have not been amply exploited. The main objectives of this work was to ascertain the antibacterial efficacy of a marine ascidian, Microcosmus exasperatus, collected from Palk Bay, Southeast coast of India, against selected urinary tract pathogens. The crude methanol extract was more active and exhibiting a broad spectrum antibacterial activity than the crude ethyl acetate extract against each of the bacterial strains tested. Maximum inhibition zone (10.0 ± 0.1 mm) was observed against the gram negative Pseudomonas aeruginosa in crude methanol extract. The consequent zone of 6.5 ± 0.1 mm was observed in ethyl acetate extract in P. aeruginosa.

Keywords: Microcosmus exasperatus, Antibacterial efficacy, zone of inhibition, urinary tract pathogens.

INTRODUCTION
In recent years, rapid use of antibiotics has resulted in the hasty evolution of multiple drug-resistant bacteria, which have caused a variety of untreatable infectious diseases. In the hope of inventing novel antimicrobial agents to control the increasing multi drug resistant pathogen. Marine organisms have received great attention during recent years for natural product chemistry, a promising new area of study. Lately, a large number of marine organisms have been reported to exhibit various kinds of bioactivities (Bakus et al., 1986;
It has been considered that the target of most antimicrobial peptides is the specific lipid bilayer of the bacterial membrane. This mechanism suggested that antimicrobial peptides might have an essential structure for attacking the bacterial membrane and retain their antimicrobial activities against resistant bacteria to conventional antibiotics, and also have minimal toxicity towards human cells (Jacob and Zaslo, 1994).

Several arguments have been put forward to explain the existence of this rich marine reservoir for bioactive substances. Marine plants and animals produce a multitude of metabolites for defense against microbial invasion and predators (Copp et al., 2003); others are involved in specific associations, showing a significant ecological importance (Aiello et al., 2000).

Among marine sources, tunicates have received more attention in the antibiotic discover. Chemical defenses are thus expected to accumulate in the tunic for adult protection. However, if defending larval stages leads to a higher survival of the species, then internal body tissues, such as gonads, are likely to be protected as well (Rhoades, 1979; Young and Bingham, 1987; Lindquist et al., 1992; Lindquist and Hay, 1996; Pisut and Pawlik, 2002). The chemistry of ascidians has become one of the most active fields of marine natural products and it has been amply demonstrated the unusual structures with significant bioactivities. Thus the present study has undertaken to ascertain the antibacterial potential of ascidian.

**MATERIALS AND METHODS**

**Collection and preparation of samples**

The specimens of *M. exasperatus* was collected from natural habitats in Mandapam, (Palk Bay) Southeast coast of India, during the low tide at a depth of 5 m in August 2013, during the low tide August 2013. Samples was transferred to the laboratory in ice cold condition and homogenized in a blender with little water and extracted with methanol and ethyl acetate at room temperature. The combined extracts were filtered and concentrated in a vacuum rotary evaporator at low temperature. The residues were weighed and dissolved in phosphate buffer saline (PBS) for further assay. All the urinary tract pathogenic bacterial strains were purchased from Raja Muthiah Medical College, Annamalai University, Tamil Nadu, India.
Antibacterial assay

Antibacterial activity was determined against, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa* using the paper disc assay method (El-Masry *et al.*, 2000) with various concentrations in triplicate (250, 500 and 1000 µg/ml). The impregnated discs were placed on the medium suitably spaced apart and the plates were incubated at 37°C for 24 h. The diameter (mm) of the growth inhibition halos caused by the tested extract was examined.

RESULTS AND DISCUSSION

The antibacterial activity of the crude methanol and ethyl acetate extract of *M. exasperatus* against different gram negative urinary tract pathogens were given in Table. 1. Methanol extract at 1000µg/ml produced a remarkable effect, 10 ± 0.1 mm against *P. aeruginosa* and 2.1± 0.2 mm in *P. vulgaris*. The corresponding zones of ethyl acetate extract produced 6.5± 0.1 mm and 3.2± 0.2 mm against *P. aeruginosa* and *P.mirabilis* respectively. In general all the extracts showed minimum activity against *P. mirabilis* and *P. vulgaris*. In 500µg/ml of methanol extract showed 4.2± 0.1 mm against *P. aeruginosa* 2.8± 0.5 mm in *P. Vulgaris* and ethyl acetate extract showed 3.6±0.1 and 2.1±0.1 against *P. aeruginosa* and *P. mirabilis*.

The test extracts showed a wide range of inhibition against *P. aeruginosa*, followed by *E. coli*, *K. pneumoniae*, *P.mirabilis* and *P. vulgaris*, in all the concentrations. All the extract of different concentration was showed a dose dependent antibacterial activity. The present investigation deals with antibacterial efficacy of the crude methanol and ethyl acetate extract of *M. exasperatus*, against gram negative human urinary tract pathogens and it was evident that the gram negative strains were more resistant. On the other hand, Abdul Jaffar Ali *et al.* (2008) reported that the maximum antibacterial activity of the crude methanol extracts of the test and mantle bodies of *Phallusia nigra* against the gram positive *Staphylococcus aureus* (inhibitory zones of 12.3±0.8 and 8.2±0.8 mm in diameter). Present study noticed that the test extract of *M. exasperatus* showed wide range of inhibition against clinical isolates, the range of inhibition varied from 2.0 ± 0.3 to 10.0 ± 0.1mm. 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 gram negative pathogens. Martinez and Baquero (2002) have reported that, nosocomial infectious bacteria exhibited least susceptibility to antibiotics and some of these bacteria out rightly developed multi drug resistance to these antibiotics. Recently from a 3 year follow up study
Dowzicky and Park (2008) reported that, Urinary tract infecting bacterial pathogens have exhibited decreased susceptibility rates to tigecycline over the years. Present results showed moderate antibacterial activity against the multi drug resistant urinary tract pathogen by *M. exasperatus*. This could be attributed that the test extract might contain secondary metabolites which inhibit the growth of bacteria. Ascidians are known to be a rich source of chemically diverse secondary metabolites with often remarkable biological activities. In many cases these compounds are simple amino acid derivatives or more complex alkaloids. They often exhibit potent anticancer activities, so they are considered as unusual cytotoxic metabolites (Vervoort *et al.*, 2000). Antibacterial and cytotoxic activity has been previously reported from extracts of some tunicates (Thompson *et al.*, 1985; Mohamed Hussain and Ananthan, 2009; Sivaperumal *et al.*, 2010; Ananthan *et al.*, 2011 b; Mohamed Hussain *et al.*, 2011). Overall, tunicates extracts caused growth inhibition in gram positive and gram negative bacteria, indicating that these extracts do not selectively inhibit one group of microorganisms (Thompson, 1985). Abourriche *et al.* (2003) evaluated the antibacterial activity against *Agrobacterium tumifaciens*, *E. coli*, *P. aeruginosae* and *S. aureus* from the extracts of Morocco Atlantic sea ascidian, *Cynthia savignyi*. It showed activity, except the dichloromethane extract, all extracts were active against bacteria. However *A. tumifaciens* was most sensitive. Activity of hexane and diethyl ether extracts of this ascidian against *A. tumifaciens* was slightly less, but higher than the activity of *Lissoclinum fragile* extracts. Tunicates have the potential to yield novel compounds of ecological, chemical and also biomedical interest (Paul *et al.*, 2008). In particular, the cosmopolitan genus *Aplidium* is renowned for the variability of its metabolites (Zubía *et al.*, 2005). A large variety of alkaloids have been isolated from this group (Arabshahi and Schmitz, 1988; Zubía *et al.*, 2005), such as piperidins, tetracyclic alkaloids and indoles, which display potent bioactivities. However, a wide range of natural products has been isolated from tunicates; little is known about the ecological roles of most of these metabolites and their allocation within ascidian tissues (Paul *et al.*, 1990, 2008; McClintock *et al.*, 1991; Vervoort *et al.*, 1998; Pisut and Pawlik 2002; Avila *et al.*, 2008). According to the optimal defense theory, defensive compounds should be located in areas that are most vital for survival and witness (Rhoades, 1979). Present study was concluded that screening of antibacterial properties from natural product such ascidians in order to find a novel antibacterial agent that can restrain the growth of bacteria in human body. Further chemical studies are being undertaken in order to isolate new antimicrobial compounds from tunicates.
Table 1. Zone of inhibition (mm diameter) of crude methanol and ethyl acetate extracts of ascidian, *Microcosmus exasperatus* against test pathogens.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration In µg/ml</th>
<th>Test pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. Coli</em></td>
</tr>
<tr>
<td>Methanol</td>
<td>250</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>7.5 ± 0.6</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>250</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>4.1 ± 0.3</td>
</tr>
</tbody>
</table>

Mean ± S.E.M(standard error of mean)

ACKNOWLEDGEMENT

The authors thank to Prof. Dr. K. Kathiresen, Dean and Director, CAS in Marine Biology, Faculty of Marine Sciences and authorities of Annamalai University for providing facilities and encouragement and the University Grants Commission (UGC) New Delhi for financial assistance.

REFERENCES


