Anti-bacterial Activity of Crude Extracts of Compound Ascidian Aplidium Multiplicatum fromVizhinjam Bay (South West Coast of India)

Divya T. Dharan[*], G. Prasad[**]

[*] Department of Zoology, University of Kerala, Kariavattom Campus, Thiruvananthapuram - 695581, Kerala, India
[**] Department of Zoology, University of Kerala, Kariavattom Campus, Thiruvananthapuram - 695581, Kerala, India.

***Abstract*-** Ascidians are rich source of bioactive agent which could be used for novel antimicrobial drugs. Ascidians are belongs to phylum chordata and class ascidiacea. In the present study a compound ascidia Aplidium multiplicatum, collected from Vizhinjam, south west coast of India was assayed for their antibacterial activity against six human bacterial pathogens. The antibacterial activity of crude extract of ascidians showed inhibitory activity against all six species. The crude methanol extract was more active exhibiting a broad spectrum antibacterial activity than the crude ethanol and acetone extract against each of the bacterial species tested. In antibacterial activity the gram negative bacteria Pseudomonas aeruginosa showed most sensitive against 12.0533± 0.010116 mm A.multiplicatum in crude methanol extract. And the minimum zone of 1.06± 0.121244 mm in K.pneumoniae in ethanol extract. The corresponding zones of ethanol extract produced a maximum zone of 10.3533± 0.7274 mm in Proteus mirabilis and acetone extract produced a maximum zone of 10.143± 0.1266 mm against Pseudomonas aureus. These results indicated that the ascidian A.multiplicatum is found to have remarkable antimicrobial activities against isolated microbes. Further, studies will fulfill for purification and structural elucidation of antimicrobial drugs.

***Index Terms*-** Ascidian, Antibacterial activity, crude extract, isolated pathogen, Vizhinjam

I. INTRODUCTION

The study of marine organisms as a source of biologically active compounds is considered a very lucrative field, having already led to the discovery of various new pharmacological tools and medicines (Bhakuni, 19941[1[], Munro et al, 1999[2] Faulkner, 2000a [3]. The work of Bergman and Feeney at the beginning of the 1950s initiated the study of marine natural products, and in the last few decades, an appreciable number of new compounds have been isolated from marine organisms (Bergman and Freeney, 1951[4] Bhakuni, 1994[1] Faulkner, 2000b[5] Faulkner, 2000c[6]. Many authors believe that the improvement in isolation and chemical identification techniques, the collaboration between chemists and pharmacologists, and most recently, the interest of pharmaceutical industries have been important determinants in the development of marine natural products research Faulkner, 2000b[5]. Ascidians, commonly called sea squirts (subphylum: Urochordata, Class: Ascidiacea) are a prolific source of diverse bioactive metabolites and also interesting organism from the view point of chemical ecology Hongwel et al, 2004[7]. The number of natural products isolated from marine organisms increases rapidly and now exceeds with hundreds of new compounds being discovered every year Proksch et al, 2002[8] Jain et al, 2008[9]. A large portion of these natural products have been extracted from marine invertebrates, especially ascidians and some of them are currently in preclinical and clinical trials Proksch et al, 2002[8]. 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.

Tunicates have been reported to be rich source of biologically active compounds and ranked third for their overall activities, next to sponges and bryozoans Davis et al, 1999[10]. Although researches on bioactive compounds from ascidians were recently initiated, it is significant that the first marine natural product Didemnin B is entering into human clinical trial and it is an ascidian metabolite. In the last two decades, the incidence of human bacterial and fungal infections has increased dramatically, in parallel with the wide spread of incurable infectious diseases associated with antibiotic –resistant bacteria. Fungal and bacterial diseases have become a growing threat, especially in immunocompromised patients, for whom few or no effective drugs are currently available Lupetti et al, 2002[11]. Accordingly, a variety of studies have been conducted in an attempt to isolate natural anti-bacterial and anti-fungal substances with potential pharmaceutical utility, and to develop and design new synthetic or semi–synthetic drugs Viejo et al, 2005[12].

The case of living marine surfaces the colonization process can additionally be affected by organic metabolites produced by the host organisms. These metabolites may affect bacteria in a number of ways, ranging from the induction of chemotactic responses to the inhibition of bacterial growth or cell death. Since they accumulate chemical defences, ascidians have been screened in a variety of pharmacological bioassays. Biological activities which have been frequently observed in ascidian crude extracts include antibiosis against both human microbial pathogens and micro organisms Mayer et al, 2007[13]. Hence a broad spectrum screening of ascidians for bioactive compound is necessary. The present study was carried out to investigate the antibacterial activity in crude extracts of ascidians from Vizhinjam bay, south west coast of India.

II. MATERIALS AND METHODS

Specimen collection and identification: Ascidians were collected as common and persistent bio foul ants from the cement blocks, pilings and pearl oyster cages of Vizhinjam bay (lat 8˚22'35.95" N-76˚59'16.40 E") , by SCUBA diving at the depth ranging from 4 to 6 m between October and November 2011. The samples were thoroughly washed with sea water, cleaned of sand, mud and overgrowing organisms at the site of collection and transported to laboratory and identified by standard study of Kott,1985[14], and Meenakshi ,2002[15].

Extraction: The extraction was followed by chellaram et al,2004[16].The freshly collected samples were weighed (20g) each and soaked in methanol; ethanol and acetone for one week and filtering through What man No.1 filter paper and the solvents were concentrated by rotary evaporator with reduced the pressure to give a dark brown gummy mass. The resultant residues were stored at 4˚Cfor further analysis.

Microbial strains used: Antibacterial activity of tissue extract was determined against six different bacterial pathogens, viz., *Klebsiella Pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi* and *Proteus mirabilis*. The clinical strains were obtained from MTCC microbial culture collection, Chandigarh.

Antimicrobial susceptibility assay: The antibacterial activity was carried out by standard disc diffusion method. The extract were applied on to 6mm sterile discs in aliquots of 30µL of solvent, allowed to dry at room temperature and placed on agar plates seeded with micro organisms. The bacteria were maintained on nutrient agar plates and incubated at 37˚C for 24 hrs. Zones of growth inhibition were measured in mm using a scale.

Statistical analysis: The results were expressed as Mean ±SD of the three independent values.

III. RESULTS

Antibacterial activity of crude methanol, ethanol and acetone extract of *A. multiplicatum* against six human pathogenic bacterial strains were presented in Table.1. Among these extracts, methanol and ethanol showed more activity against all pathogens than acetone extract. In the present investigation, methanol extract of *A. multiplicatum* showed high antimicrobial activity against both gram positive and gram negative bacteria. From the bacteria tested *P. aeruginosa* was the most sensitive against methanol extract 12.0533± 0.10116 mm of *A.multiplicatum*. And the minimum zone of 2.166± 0.13081 mm was observed in *S. typhi. Corresponding zones of ethanol extract produced a maximum zone of 10.3533± 0.7274 mm in P. mirabilis and minimum zone of 1.06± 0.121249*

Table 1. Antibacterial activity of Aplidium multiplicatum against human pathogens

PATHOGENS	METHANOL	ETHANOL	ACETON
Staphylococcus aureus	3.9833±0.035119	3.2733±.24986	2.9667±0.035119
Salmonella typhi	2.1466±0.13081	2.1466±0.1350512	1.97±.03
Klebsiella pneumonia	0	1.06±0.121244	0
Pseudomonas aeruginosa	12.0533±0.10116	10.3533±0.72748	10.143±0.1266
E.coli	5.15833±0.14682	7±0.1	5.9566±0.058595
Proteus mirabilis	4.0733±0.2289	8.0633±0.118462	4.0933±0.17039

Zone of inhibition* (mm)

*Zone in mm indicates the distance from the border of the disc to the edge of the clear zone

mm in K. pneumonia. Acetone extract produced a maximum zone of 10.143± 0.1266 mm against P. aureus and minimum activity 1.97± 0.03 mm against S. typhi. There was no activity was observed in acetone and methanol extract against K. Pneumonia. Both extracts showed a broad-spectrum of anti-bacterial activity against, S. aureus, S. typhi, P. aeruginosa, E. coli and P. mirabilis.

IV. DISCUSSION

Marine organisms have been found to produce a great diversity of novel bioactive secondary metabolites and be potential source of drug discovery. Extensive investigations of ascidians pharmacology research have been undertaken all over the world. Several drug discovery projects have screened

ascidians for antibiotic activities. Overall, ascidian extract caused growth inhibition in gram positive and gram negative bacteria, indicating that these extracts do not selectively inhibit one group of micro organisms Thompson et al, 1985[17]. Here we examined antibacterial activity of the crude methanol and ethanol extracts of A. multiplicatum against gram positive and gram negative bacteria and it was evident that the gram negative strains were more resistant, than gram positive. This study is contrary with the findings of Ali et al, 2008[18] who reported maximum antibacterial activity of the crude methanol extract of the test and mantle bodies of P. nigra against the gram positive strains inhibitory zones of (12.3± 0.8 mm) and (8.2± 0.8 mm) respectively.

In the present study A. multiplicatum showed promising source of antibacterial activity in crude extracts. It showed high antibacterial activity against six pathogens assayed, from the bacteria tested: P. aureus was the most sensitive against methanol extract (12.0533± 0.10116 mm). Minimum zone of inhibition (2.166 ± 0.13081 mm) was observed in S. typhi against methanol extract. The crude ethanol extract showed maximum activity against P. mirabilis (10.3533± 0.7274 mm) followed by; (8.0633± 0.118462 mm) in P. aureus , (7± 0.1 mm) in E. coli,(3.2733± 0.24986 mm) in S. aureus, and (2.14667± 0.130512 mm) in S. typhi respectively. And minimum activity was noticed against (1.06± 0.12124 mm) in K. pneumoniae. Acetone extracts showed maximum activity against (10.143± 0.1266 mm) in P. aureus, followed by zone of inhibition (5.9566± 0.058595 mm) in P. mirabilis, zone (4.0933± 0.17309 mm) against E. coli, (2.9667± 0.03511 mm) in S. aureus and minimum zone of (1.97± 03 mm) in S. typhi. No activity was observed in both methanol and acetone extract against K. pneumoniae. The result of present study similar to that the previous report of Sivaperumal et al, 2010[19] who reported that the methanol extract of A. multiplicatum exhibited antimicrobial activity against most of the bacterial species studied; no effect was observed in K.pneumoniae species.

The crude methanol extract of Policlinium madrasensis and Phallusia arabica were found to have higher antibacterial activities against P. aeruginosa Amutha et al, 2010[20]. In this study also P. aeruginosa was the most sensitive to methanol extracts of A. multiplicatum than ethanol and acetone. Ramasamy et al, 2003[21] also reported that the P. aeruginosa was the most successful bacteria for all fractions of ascidian extracts with maximum zone of 8-5mm. The bacterial species like Bacillus and Pseudogeneus species found to have inhibitory effect for the extracts of colonial ascidians with MIC value 200mg/ml Sivaperumal et al,[19] .Kartykayen et al, [22] reported that the methanol and ethanol extracts of ascidian showed more activity against all pathogens than hexane and butanol extracts .The present study reported that methanol and ethanol extracts of A. multiplicatum showed higher activity against microbes than acetone extract .The crude methanol, ethanol and acetone extracts of A.multiplicatum was more effective against gram negative bacteria than gram positive bacteria. This study similar to the previous report of Sivaperumal et al, 2010[19] reported that the crude ethyl acetate of A. multiplicatum was more effective against gram negative bacteria than gram positive bacteria.

Antibacterial activity has previously been detected in methanol and dichloromethane extracts of the ascidians H. pyriformis and a mixture of two Styela species where one of the species was S. rustica Lippert et al, 2003[23]. Prem Anand and Edward 2002[24] reported that comparatively ascidians D. pasmathodes seems to be promising source of antibacterial compound. Ramasamy et al, 2003[21] reported that for the crude methanol extract of D. pasmathodes, the range of inhibition of bacteria varied from 6 to 10 mm with an average of 7.1 mm. Meenakshi, 2002[15] revealed that the preliminary screening of nine species of ascidian indicate, the presence of antibacterial activity of the three different solvent and methylene extract showed maximum activity followed by methanol and hexane. Methanol and methylene chloride extracts of Aplidium indicam were active against all pathogens. The test body of P. nigra harboured smaller number of total heterotrophic bacteria compared to that of the surrounding water medium Ali et al, 2008[18].

The tunicates have the potential to yield novel compounds with ecological, chemical, and biomedical interest Paul et al, 2008[25]. In particular, the cosmopolitan genus Aplidium is renowned for the variability of its metabolites. A large variety of alkaloids have been isolated from this group, such as piperidins, tetracyclic alkaloids and indoles, which display potent bioactivities Zubia et al, 2005[26]. Many studies have been conducted to examine the antimicrobial activity of ascidians against bacteria, fungi even tumour cells Ronald et al, 1997[27]. The extracts from D. pasmathodes showed the promising results against isolated and human pathogens. These results indicate that ascidians exhibits remarkable activity against microbes Kumaran et al, 2011[28]. The continuing and over whelming contribution of ascidian metabolites to the development of new pharmaceuticals are clearly evident and need to be explored. Antibacterial compounds form natural resources would be alternative to overcome the resistance problems. Thus the current studies revealed the presence of antibacterial activity from ascidians of Vizhinjam bay South west coast of India has much importance in marine secondary metabolites. Further, purification of the actual compounds involved in the activity may lead to the discovery of novel antimicrobial compounds.

V. CONCLUSION

Activities found in crude extracts showed promising results and with enormous potential for discovery and development and marketing of novel marine bio products methods by which these products can be supplied in a way that will not disrupt the ecosystem or deplete the resources. It is worthy to note that the product from nature source is good for health and devoid of side effects. However, further investigations involving application of the extracts as drug for human administration need more research.

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AUTHORS

First Author – Divya T. Dharan, Department of Zoology, University of Kerala, Kariavattom Campus, Thiruvananthapuram - 695581, Kerala, India, Email: dvyremya@yahoo.co.in
Second Author – G. Prasad, Department of Zoology, University of Kerala, Kariavattom Campus, Thiruvananthapuram - 695581, Kerala, India, Email: dvyremya@yahoo.co.in