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Antibacterial Potential of Marine Ascidian *Phallusia arabica* Against Isolated Urinary Tract Infections Bacterial Pathogens

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ABSTRACT

In-vitro antibacterial activity of marine ascidian *Phallusia arabica* was investigated against urinary tract infection bacterial pathogens using disc diffusion method. The crude ethyl acetate extract was more active exhibiting a broad-spectrum antibacterial activity than that of the crude methanol extract against each of the microbes tested. Maximum inhibition zone (12 mm) was observed against *Proteus mirabilis* in crude ethyl acetate extract (1 mg mL⁻¹ concentration) and the minimum inhibition zone (1.5 mm) was observed against *Pseudomonas aeruginosa* in methanol extract (1 mg mL⁻¹ concentration). The range of MICs and MBCs were high in ethyl acetate extract and it was low in the methanol extract. This result suggest that *P. arabica* can be used as effective inhibitor of urinary tract infection pathogens making them applicable to medical devices and antimicrobial control systems.

Key words: Antibacterials, ascidians, urinary tract infections pathogens, minimum inhibitory concentration, minimum bactericidal concentration

INTRODUCTION

Patients with non-infectious disease who have stay in hospital have high risk to acquire nosocomial infection. It has been reported that 10% hospital patients acquire this infection while staying in hospital (Asefzadeh, 2005). The common pathogenic bacteria which include *Escherichia coli*, *Klebsilla pneumoniae*, *Haemophilus influenza*, *Streptococcus pneumoniae* and *Proteus vulgaris*, are the major causative agents of nosocomial infections. Generally, nosocomial infections develop in urinary tract (Saonuam *et al.*, 2008). Ocean has potent bioactive compounds isolated from marine organisms which are currently used as food. This mechanism has proved to be timely alternative natural medicine to human beings. The bioactive compounds from ascidians was recently initiated, it is significant that the first marine natural product entering in to human clinical trials, didemnin B, is an ascidian metabolite. Tunicates have been reported to be rich sources of biologically active compounds and ranked third for their overall activities, next to sponges and bryozoans (Davis and Bremner, 1999). Actinobacteria, the filamentous bacteria have provided many important bioactive compounds of high commercial value and are being routinely screened for new bioactive substances (Siva Kumar *et al.*, 2011). The first systematic search for antibiotics resulted in the discovery of Actinomycetin from Actinomycete bacteria (Sultan *et al.*, 2002). A large proportion of 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 bioactive substance which possesses potent anticancer activity Ecteinascidin-743 was isolated from Caribbean Sea squirt *Ecteinascidia turbinata* (Russo *et al.*, 2008). Such potential ascidians need to be explored for the pharmaceutical purpose. The study was carried out to establish the occurrence of antibacterial activity of ascidians extracts, collected from Southeast coast of Tamil Nadu, India

MATERIALS AND METHODS

Collection and preparation of samples: The ascidian *Phallusia arabica* (Chordata: Ascidiacea: Pleurogona: Pyuridae) were collected during the low tide of the intertidal area at Thoothukudi coast, Tamil Nadu, Southeast coast of India, between 20 and 23 August 2010. The collected samples were rinsed with sterile seawater to remove associated debris and salt. The samples were weighed (10 g) and preserved separately in methanol and ethyl acetate (1:2) and brought to the laboratory. Samples were then soaked in the above mentioned solvents for 48 h, 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 was centrifuged at 3000 rpm. The solvent was evaporated under reduced pressure using desiccator and the residue was weighed and dissolved in dimethyl formamide for using them for the antibacterial activity. All the urinary tract infection bacterial pathogen strains were obtained from Raja Muthiah Medical College, Annamalai University.

Antibacterial susceptibility assay: Ascidian crude extract was tested for inhibition of bacterial growth against urinary tract infections bacterial pathogens. The extract of *P. arabica* was tested for antibacterial activity at two different concentrations by disc diffusion method (Avelin *et al.*, 1991).

Minimum Inhibitory Concentration (MIC): Minimum inhibitory concentration was determined by the following procedure (Collins *et al.*, 1995).

Minimum Bactericidal Concentration (MBC): Minimum bactericidal concentration was experimented after the MIC in freshly prepared agar plates, followed by standard method of Alade and Irobi (1993).

RESULTS AND DISCUSSION

In vitro antibacterial screenings of marine ascidians against selected clinical isolates were performed and the inhibition zones of the extract against the specific test organisms were given in Table 1. Maximum inhibition zone (12 mm) was observed against *P. mirabilis* in crude ethyl acetate extract (1 mg mL⁻¹ concentration) of *P. arabica* and the minimum inhibition zone (1.5 mm) was observed against *P. aeruginosa* in methanol extract (1 mg mL⁻¹ concentration). This view is consistent with the findings of Anand and Edward (2002) reported that ethyl acetate crude extract of ascidian exhibited strong antibacterial activity than methanol extract and Petroleum ether of all the strain tested. The methanol crude extract of *P. Arabica*, the range of inhibition of the clinical isolates of the bacteria varied from 1.5-6 mm was observed in both (500 µg mL⁻¹ and 1 mg mL⁻¹) concentration. This result is lesser than the findings of Hussain and Ananthan (2009), who reported that the crude methanol extract of *Didemnum psammathodes*, the range of inhibition of the bacteria varied from 2-15 mm with an average of 4.3 mm. On the other hand, Ramasamy and Murugan (2003) have reported that the crude methanol extract of *Didemnum psammathodes*, the

range of inhibition of the bacteria varied from 6 and 10 mm with an average of 7.1 mm. The Ethyl acetate extract of *T. clinids*, the range varied between 1 and 8.5 mm with an average of 3.18 mm.

The crude ethyl acetate extract of *P. arabica* showed the maximum antibacterial activity in (1 mg mL⁻¹ concentration) against *P. mirabilis*, followed by *E. coli*, *P. vulgaris*, *Klebsiella* sp and *P. aeruginosa* and the minimum activity was noticed in all 500 µg mL⁻¹ concentration of the same extract. The crude methanol extract of *P. arabica* showed the maximum antibacterial activity in (1 mg mL⁻¹ concentration) against *P. mirabilis*, followed by *P. vulgaris*, *E. coli*, *Klebsiella* sp. and *P. aeruginosa*. There is no activity against all 500 µg mL⁻¹ concentration methanol extract (Table 1).

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs) of the crude extracts were shown in Table 2. The range of MIC varied between 0.25-1.85 (500 µg mL⁻¹ and 1 mg mL⁻¹ concentrations) against all the bacterial strains used in this study and MBC ranges between 0.20-2.10 (500 µg mL⁻¹ and 1 mg mL⁻¹ concentrations) against all the bacterial strains. Ascidians are already reported for rich nitrogenous source with a wide range of biological activities (Biard *et al.*, 1994). The range of MICs is highest in ethyl acetate extract 1.85 (1 mg mL⁻¹ concentration) and methanol extract 1.05 (1 mg mL⁻¹ concentration). Range of MBCs was high in ethyl acetate extract 2.10 (1 mg mL⁻¹ concentration) and methanol extract 1.15 (1 mg mL⁻¹ concentration). The range of MICs was low in the ethyl acetate extract 0.25 (500 µg mL⁻¹ concentration) and methanol 0.25 (1 mg mL⁻¹ concentration). The range of MBCs was low in ethyl acetate extract 0.20 (500 µg mL⁻¹ concentration) and methanol 0.60 (1 mg mL⁻¹ concentration). On the other hand, Natarajan *et al.*, (2010) reported that the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MBC) of the methanolic extract of *Polyclinum madrasensis*, MIC varied between 0.70-0.95 mg mL⁻¹ and MBC ranges between 0.85-1.10 mg mL⁻¹ against all the bacterial strain tested.

Table 1: Antibacterial activity of *Phallusia arabica*

Microorganisms	Inhibition zone (mm)			
	Ethyl acetate		Methanol	
	500 µg mL ⁻¹	1 mg mL ⁻¹	500 µg mL ⁻¹	1 mg mL ⁻¹
<i>E. coli</i>	5	7	-	4
<i>Klebsiella</i> sp.	-	5	-	4
<i>P. mirabilis</i>	8.5	12	-	6
<i>P. vulgaris</i>	2	6	-	5
<i>P. aeruginosa</i>	2	4	-	1.5

-: No activity was observed

Table 2: MIC and MBC of different concentration crude extracts of *Phallusia arabica*

Microorganisms	Ethyl acetate				Methanol			
	MIC (500 µg mL ⁻¹)	MIC (1 mg mL ⁻¹)	MBC (500 µg mL ⁻¹)	MBC (1 mg mL ⁻¹)	MIC (500 µg mL ⁻¹)	MIC (1 mg mL ⁻¹)	MBC (500 µg mL ⁻¹)	MBC (1 mg mL ⁻¹)
<i>E. coli</i>	0.25	0.70	0.35	0.65	-	0.80	-	1.05
<i>Klebsiella</i> sp.	-	0.85	0.25	0.95	-	1.05	-	1.15
<i>P. mirabilis</i>	0.35	1.85	0.45	2.10	-	0.80	-	0.75
<i>P. vulgaris</i>	0.30	0.85	0.40	0.90	-	0.85	-	1.10
<i>P. aeruginosa</i>	0.25	0.55	0.20	0.50	-	0.25	-	0.60

-: No activity was observed

Shobu and Pawlik (2007) reported that crude organic extracts of whole tunic and internal tissues of *P. nigra* contained vanadium metabolites (225 and 750 ppm dry mass, respectively) and were palatable to blue head wrasse, *Thalassoma bifasciatum*. Crude extracts also exhibited no antimicrobial effects against a panel of four marine bacteria (*Vibrio parahaemolyticus*, *Vibrio harveyi*, *Leucothrix mucor* and *Deleya marina*). Jaffarali *et al.* (2008) reported that the test of *P. nigra* exhibited a bactericidal effect, with the zone of inhibition ranging from 2.3 to 12.3 mm against all pathogens tested, as referred to the mantle body. This result more or less consistent to our results. Dowzicky and Park (2008) reported that UTI bacterial pathogens have exhibited decreased susceptibility rates to tigecycline over the years. Marine genus synthesizes active constituents which are used in traditional and complementary medicine (Anbu-Jeba-Sunilson *et al.*, 2009). Antibacterial compounds from natural resources would be the alternative to overcome the resistance problem. Since, the present study indicated that the crude extract of *P. arabica* showed antibacterial activity which could be used in pharmacological research.

CONCLUSION

Present study deduce that the continuing and overwhelming contribution of ascidians metabolites to the development of new pharmaceuticals are clearly evident and need to be explored. After taking in to consideration the immense side effects of synthetic drugs, great attention has to be paid for the discovery of novel drugs from marine natural products.

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