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ANTI MICROBIAL ACTIVITY OF A SIMPLE ASCIDIAN, *PHALLUSIA NIGRA*

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ABSTRACT

Marine organisms produce a huge number of secondary metabolites, perhaps to a larger expense than observed in terrestrial ecosystems. Ascidians are marine sedentary organisms. Ethanolic extract of the simple ascidian, *Phallusia nigra* Sav. has been tested against ten bacterial and three fungal pathogens by using disc diffusion method. The antimicrobial effects of animal extract against the different strains are presented and it is found that the inhibitory effect was proportional to concentration gradient.

KEYWORDS: *Phallusia nigra*, antibacterial, antifungal, disc diffusion, MIC.

INTRODUCTION

Ocean is the mother of life. Many marine organisms have been screened over the past two decades. Among the marine organisms studied, sponges occupy the leading place followed by ascidians coming under the class Ascidiacea. Didemnin, isolated from colonial ascidian *Didemnum* species was the first compound to enter clinical trials for the treatment of cancer. Sedentary animals are loaded with rich secondary metabolites acting as defense chemicals. It has been widely demonstrated that the ascidians are rich in bioactive substances [1]. These metabolites may affect bacteria in a number of ways ranging from the induction of a chemotactic response to the inhibition of bacterial growth or cell death [2,3].

Ascidians are marine invertebrates which ranks second with promising source of drugs [4]. Ascidians or sea squirts are cosmopolitan, exclusively marine invertebrates which constitute a rich source of biologically active secondary metabolites [5]. Most of the ascidians are utilized as food in various countries and they are known to produce bioactive metabolites which

prevent bio-fouling and this can be considered as a kind of autogenic protection [6]. The number of natural products isolated from marine organisms increase rapidly and now exceeds with hundreds of new compounds being discovered every year [7,8]. The potential of ascidians as a source of biologically active product on virulent hospital isolates is largely unexplored. Hence in the present investigation the antimicrobial activity of the ethanolic extract of the whole animal of *Phallusia nigra* has been carried out on five gram positive and five gram negative bacteria and three fungal pathogens isolated from hospital samples by using zone of inhibition method. The antibiotics Ofloxacin and Nystatin were used as standard for bacteria and fungi respectively.

MATERIALS AND METHODS

Animal material

Samples of *Phallusia nigra* (Family: Ascidiidae) were collected from Tuticorin coast by SCUBA diving. They were identified and authenticated by Dr. V.K. Meenakshi, Associate Professor, Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin - 628002. A Voucher specimen AS-2083 has been deposited in the National Collections of Ascidians in the Museum of the Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin-628 002.

Preparation of extract

Epibionts adhering to the test were carefully removed, washed with sterile sea water, dried under shade and homogenized to get a coarse powder. The coarse powder was stored in an airtight container and used for further investigations.100g of powdered animal material was successively extracted with solvents like petroleum ether (40^0-60^0C), benzene, chloroform, ethanol and water using a soxhlet apparatus. The extracts were cooled to room temperature, evaporated in a rotary evaporator under reduced pressure to obtain a brown sticky residue which was used for antimicrobial assay.

Microbial strains used

Antibacterial activity was determined against eleven different bacterial pathogens, five gram positive bacteria, *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Sarcina lutea* and *Staphylococcus aureus,* six gram negative bacteria, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas pyocyanus* and three fungi, *Candida albicans, Aspergillus niger* and *Saccharomyces cerevisiae.* These clinical

strains were obtained from the hospital samples of the Department of Microbiology, P.S.G. Medical and Research Institute, Coimbatore 641 004, Tamilnadu, India.

Preparation of test micro organisms

A loopful of the test organism was transferred to already sterilized 10 ml Nutrient agar and incubated overnight at 37^0C for bacteria and 30^0C for fungi. *Aspergillus niger* was cultured as a slant culture in an acidified PDA (Potato Dextrose Agar) media. 25 ml of sterilized Muller-Hinton Agar (MHA) (Hi Media, Mumbai, India) was poured in petriplates and allowed to solidify at room temperature on which the test organisms were inoculated.

Antimicrobial assay

The antimicrobial activity was measured by Disc Diffusion method [9]. The sterile discs were impregnated with the known concentration of the various extracts (15 µl) and standard drugs. The discs were then placed on the already inoculated petridishes containing the inoculum of test microbes in such a way that there is no overlapping of the zones of inhibition. The seeded plates were then incubated at 37^0C for 24 hours and 48 hours for bacteria and fungi respectively. The antimicrobial activity of the animal extracts was recorded as the mean diameter of the resulting inhibition zone of growth measured in millimetres.

From the results, the Active Index (AI) and Proportion Index (PI) were calculated using the following formulae,

$$\text{Active Index (AI)} = \frac{\text{Inhibition zone of the test sample}}{\text{Inhibition zone of the standard}}$$

$$\text{Proportion Index (PI)} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

RESULTS AND DISCUSSION

Antimicrobial activity of three different concentrations of ethanol extract of *Phallusia nigra* has been evaluated *in vitro* against gram positive and gram negative bacteria and fungal pathogens. The results of antibacterial and antifungal activity have been presented in the Table 1.

Table 1: Antimicrobial activity of the ethanol extract of *Phallusia nigra* Sav.

Name of the organism	Zone of Inhibition (mm)										
	Petroleum ether (40^0-60^0C)		Benzene		Chloroform		Ethanol		Water		Standards
	DIZ*	AI #	DIZ*	AI #	DIZ*	AI#	DIZ*	AI#	DIZ*	AI#	
Bacillus Cereus	-	0	-	0	-	0	5	0.42	6	0.50	8[a]
Bacillus subtilis	-	0	-	0	-	0	-	0	1	0.06	16[a]
Bacillus megaterium	-	0	-	0	-	0	14	1.08	15	1.15	12[a]
SarcinaLutea	-	0	-	0	-	0	9	0.75	12	1.00	14[a]
Staphylococcus aureus	-	0	-	0	-	0	20	1.17	14	0.82	17[a]
Escherichia coli	-	0	-	0	10	0.62	19	1.12	16	0.94	17[a]
Pseudomon asaeruginosa	-	0	-	0	3	0.19	22	1.37	17	1.06	16[a]
Klebsiella pneumonia	-	0	-	0	-	0	22	1.15	20	1.05	19[a]
Salmonella typhi	-	0	-	0	6	0.38	19	1.19	22	1.38	16[a]
Pseudomonas pyocyanus	-	0	-	0	-	0	12	0.84	4	0.29	10[a]
Candida albicans	-	0	-	0	-	0	25	1.08	25	1.09	24[b]
Aspergillus niger	-	0	-	0	-	0	25	1.25	28	1.40	20[b]
Saccharomyces Cerevisiae	-	0	-	0	-	0	24	1.13	22	1.04	20[b]

*DIZ- Diameter of zone inhibition; #AI- Active Index

a- Ofloxacin; b- Nystatin; - No inhibitory effect.

Table 1, shows that petroleum ether (40^0-60^0C) and benzene extracts did not show inhibitory effect against any of the pathogens under investigation. Chloroform extract showed low inhibitory effect on *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.* Highly significant activity was noticed in ethanol and water extracts against both bacteria and fungi. Hence *in vitro* antibacterial screening of the ethanolic extract of *Phallusia nigra* against selected clinical isolates were performed and the inhibition zones of the extract against the specific test organisms were given in Table 1. Among the ten bacteria tested, ethanolic extract of *P. nigra* was more sensitive against *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Aspergillus niger* and *Saccharomyces cerevisiae.* Also the ethanolic extract showed highly significant activity against all the gram negative bacterial strains. Fungal strains were found to be highly sensitive to the ethanolic extract of *Phallusia nigra* when compared to that of the bacterial strains. This view is contrary with the findings of Abdul Jaffar Ali et al. (2008) who reported.[10] the maximum antibacterial activity exhibited by the Gram positive bacteria than in Gram negative bacteria of crude methanol extracts of the test and mantle bodies of *Phallusia nigra*. It is clearly evident that the antibacterial activity has been previously reported from extracts of some ascidian extracts

caused growth inhibition in gram positive and negative bacteria. Organic substances isolated from the marine plants and animals have been shown to affect bacterial behaviour as reported by Bell and Mitchell [11]. GC-MS study of the methanol extract of *Phallusia nigra* revealed the presence of alcoholic compounds such as dl-3,4 dimethyl-3,4-hexanediol, dl-6-methyl-5-hepten-2-ol and 2-methyl-3-decanol showing antimicrobial activity [12]. Hence it may be concluded that these alcoholic compounds may be responsible for the potent antimicrobial activities of *Phallusia nigra*.

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