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Cytotoxicity of the crude extracts of Marine ascidians (Tunicata: Ascidiacea) from Tuticorin, Southeast coast of India

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ABSTRACT

Marine organisms such as ascidians, sponges and soft corals, containing symbiotic microorganisms are a rich source of bioactive compounds. This study examined the cytotoxic potential of two ascidians found in the coastal waters of Tuticorin. The ascidians samples Didemnum psammatode and Phallusia arabica were extracted in methanol and ethanol. The crude extracts were tested for cytotoxicity using the brine shrimp lethality assay. The crude methanol extract of compound ascidian indicated the highest activity with LC_{50} value of (106.965 µg/ml) and the ethanol extract of simple ascidian showed the lowest activity with LC_{50} value of (227.055 µg/ml). Further studies are necessary for a better characterization of the active principles of these extracts and a possible elucidation of the mechanisms of action.

Keywords: Ascidian, *Didemnum psammatode, Phallusia arabica*, Cytotoxicity, Brine shrimp.

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 [1, 2, 3]. Marine organisms such as ascidians, sponges and soft corals, containing symbiotic microorganisms are a rich source of bioactive compounds [4, 5, 6]. Some biologically active metabolites present in marine organisms have pharmaceutical potential to cure diseases such as cancer. One promising group of bioactive metabolites is ecteinascidins, biosynthesized by the colonial ascidian *Ecteinascidia turbinata* (Herdman), which have strong antitumor properties [7, 8]. 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 [9, 10, 11].

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 [10]. Studies performed at the United States National Cancer Institute have shown that marine invertebrates show a higher incidence of cytotoxic compounds than any other zoological group [2]. Sponges, bryozoans, and tunicates are among the most promising organisms as sources of new active principles for drug development [11].

MATERIALS AND METHODS

Specimen Collection and Identification: Bulk samples of ascidians, *D. psammatode* (Sluiter, 1895) and *P. arabica* (Savigny, 1816) were collected from Tuticorin coast (Lat 8° 47’ 20” and Long. 78° 09’ 70”), India by SCUBA diving at a depth ranging from 4 to 6 m between August and September, 2010. The samples were thoroughly washed with sea water and cleaned of sand and overgrowing organisms at the site of collection and transported to laboratory.

Extraction: The extraction was followed by [12].The freshly collected tunicates were soaked in methanol and ethanol at the site of collection until workup. The extracts were filtered through Whatman®No.1. Filter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rotavapor® at 30°C) with reduced pressure to give predominantly an aqueous suspension and concentrated under reduced pressure to give a dark brown (10.58gms) and yellow gummy mass (12.32gms) respectively. The crude extracts were used for biological activities.

Brine shrimp assay

Brine shrimp (*Artemia salina*) eggs were hatched in a beaker filled with filtered sea water under constant aeration. After 48 h, the phototrophic nauplii were collected by pipette. The nauplii were counted macroscopically in the stem of the pipette against a lighted background. Ten shrimp were transferred to each well of 24-multiwell plates containing the samples. The plates were maintained under illumination. Survivors were counted after 24 h of incubation and the percentage of deaths at each dose and controls (seawater) were determined [13]. The crude extracts were dissolved in 0.01 ml of DMSO and incorporated into 5 ml of sea water (pH= 8.8 and Salinity = 28 ‰) containing ten *Artemia salina*. Each concentration (50, 100, 150 and 200 µg/ml) was tested thrice and a control (saline water) was done each time. The LC_{50} values of brine shrimp were obtained from counts using the prohibit analysis method described by [14].

RESULTS

The crude extracts of both *D. psammatode* and *P. arabica* are showing cytotoxic properties against *Artemia salina* larvae. The brine shrimp assay is considered as a reliable indicator for the preliminary assessment of toxicity. This assay is widely employed in the screening process for the isolation of bioactive metabolites. In the present study, both extracts have been tested at (50, 100, 150 and 200 µg/ml) different concentration, indicating the presence of cytotoxic compounds in these ascidians. The LC_{50} values are given in (Table 1). The crude methanol extract of compound ascidian indicated the highest activity with LC_{50} value (106.965 µg/ml) and the ethanol extract of simple ascidian exhibited weaker brine shrimp lethality with LC_{50} value of

(227.055 µg/ml). The compound ascidian exhibited wide range of cytotoxicity than the simple ascidian.

Table 1. Cytotoxicity of Ascidians extracts against *Artemia salina* larvae

Concentration(µg/ml)	*P. arabica*		*D. psammatode*	
	Methanol	**Ethanol**	**Methanol**	**Ethanol**
Control	5	5	0	0
50	19.4±1.6	16.3±0.8	24.0±1.2	18.0±0.9
100	28.3±0.6	19.4±1.0	38.5±0.6	27.4±0.7
150	42.3±0.8	35.6±0.9	56.3±0.4	47.8±0.8
200	63.7±0.4	52.7±0.7	87.8±0.9	65.3±1.1
LC_{50}	**174.779**	**227.055**	**106.965**	**151.881**

Mean± SE Standard Error

DISCUSSION

The studies conducted with marine natural products during the last decades has uncovered many substances with biomedical potential, which has raised the interest of many research groups toward this ecosystem as a source of new drugs [2]. Several cytotoxic alkaloids have been isolated from ascidian *Eudistoma* sp., such as eudistomins, eilatin, staurosporine derivatives, methyleudistomins, and pibocin [15, 16, 17, 18, 19]. These alkaloids have different degrees of *in-vitro* cytotoxicity against various tumor cell lines, and they are considered potential lead compounds for the development of new chemotherapeutic agents. The extract derived from *Euherdmania* sp. produced results similar to those with *E. vannamei* in the different bioassays although, in all cases, showing a lower potency [20].

D. psammatode and *P.arabica* had not been previously investigated for the presence of cytotoxic compounds. Hence the present investigations have been carryout to study the cytotoxic potential of these species. The crude methanol extract of compound ascidian indicated the highest activity and the ethanol extract of simple ascidian exhibited weaker brine shrimp lethality than compound ascidian. The methanol extract showed highest activity compare to ethanol extract in the brine shrimp assay. On the other hand *Euherdmania* sp. extract exhibited a LC_{50} of 77.8 µg/ml in the brine shrimp assay [20]. The presence of cytotoxic alkaloids in *Cystodytes dellechiajei* such as ascidemin and cystodytins D-I [21, 22].

CONCLUSION

We concluded that the ascidians are a rich source of compounds with cytotoxic proprieties; these marine natural products can be used for the discovery and development of novel hemotherapeutic agents.

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