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Biological activities of marine sponge *Callyspongia diffussa* (Ridley, 1884) collected from Mandapam coast

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Abstract

This work reports the results of a screening programme based on marine sponge extract from the Southeastern Mandapam coast. Sponge species was collected by scuba diving in rocky shores, from 2010, were screened for haemolytic, neurotoxic and antibacterial activities. Methanolic extract of the sponge species was obtained and the crude extract of *Callyspongia diffusa* induced haemolysis (32 HU/g and 16 HU/g) in chicken and goat erythrocytes. The extract of *C. diffusa* showed specific neurotoxic activity on sea shore crab. The screening for antibacterial activity showed that the methanolic extract of sponge was active against ten species of bacterial pathogens. The maximum zone of inhibition was 10mm against *Pseudomonas aeuruginosa* and *Salmonella paratyphii* while the minimum inhibition zone was 7mm, against *Salmonella typhii* and *Klebsiella pneumoniae* respectively.

Keywords: Sponge, Palk Bay Antibacterial, Toxic, activity, Haemolytic.

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INTRODUCTION

Marine organisms including those from coral reef ecosystems have become sources of great interest to natural product chemistry, since they provide a large proportion of bioactive metabolites with different biological activities (Faulkner, 2000). Sponges (Phylum: Porifera) are the most primitive multicelled animals that have existed for 700-800 million years. Of the approximately 15,000 sponge species, most of them occur in marine environments. Only about 1% of the species inhabits fresh water (Belarbi *et al.*, 2003). Majority of the marine natural products have been isolated from sponges, coelenterates (sea whips, sea fans and soft corals) tunicates, opisthais branch molluscs, echinoderms, sea grass, bryozoans and wide variety of marine micro organisms in their tissues (Prakash Williams *et al.*, 2007).

Marine sponges are the rich source of structurally unique natural compounds, several of which have shown a wide variety of biological activities (De Rosa *et al.*, 2003). It is well known that even excellent drug candidates from sponges are often not developed because those sponges are rare, difficult to collect or both. Sponges harbour rich diversity of microorganisms in their tissues and in some cases constitute up to 40% of the biomass, as in the case of the Mediterranean sponge *Aplysina aerophoba* (Friedrich *et al.*, 1999). Numerous natural products from marine invertebrates show striking structural similarities to metabolites of microbial origin, suggesting that microorganisms are the true source of these metabolites or are intricately involved in their biosynthesis (Proksch *et al.*, 2002). Convincing evidence for the involvement of microorganisms in natural product synthesis has been complied for the tropical sponges *Dysidea herbacea* and *Theonella swinhoei*, in which the producing microbe is a cynobacterium in the former and a bacterium in the latter (Proksch *et al.*, 2002). Thus an alternative strategy targeting the microorganisms associated with sponges for the screening of bioactive natural products may prove to be an effective approach to circumvent the associated difficulties of dealing with the organism itself.

Among marine invertebrates, the sponges continue to be a rich source of novel secondary metabolites, with a diversity of biological activities that continue to inspire the efforts of synthetic organic chemists. They are even considered to be the more prolific producers of new marine natural products. Until now, more than 5000 different compounds have been isolated from about 500 species of sponges (Muller *et al.*, 2004). Marine sponges are wealthy sources of bioactive compounds some of them are precursors for new pharmacological tools and medicine. The aim of this work is to study the haemolytic activity and antibacterial activity of the extract of the marine sponge *Callyspongia diffusa*.

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MATERIALS AND METHODS

Collection of Sponge

Colonies of marine sponge *Callyspongia diffusa* (Class: Demospongiae) was collected from Mandapam coastal water (Lat. 9° 17′ N Long. 79° 17′), Southeast coast of India by SCUBA diving at a depth of approximately 5 meters during January 2010. The sponge samples were placed inside sterile ethyl polythene bags underwater and transferred to the laboratory, where they were subjected to extraction with methanol.

Preparation of organic extract

Sponge specimens (100 g) were cut into small pieces and placed in 200 ml mixture of methanol: dichloromethane (1:1). After 24 h, the extract was decanted and collected for analysis. Following triplicate extraction, the extracts were pooled, filtered through Whatman® No.1. filter paper and concentrated in a rotary evaporator (R-200 Buchi Rotavapor® at 30 °C). The resultants were stored at 4 °C for further use.

Preparation of erythrocyte suspension

Haemolytic activity of the crude toxin was tested by the method of Pani Prasad and Venkateshwaran (1997). The blood was collected from the goat and chicken and 2.7% EDTA was used as anticoagulant at 5% of the volume of blood. The blood was centrifuged at 5,000 rpm for 7 minutes with normal saline (pH 7.2). The supernatant was discarded and the RBC pack was resuspended in normal saline. This process was repeated thrice and finally the concentrate thus obtained from the final RBC pack was used to prepare 1% RBC suspension.

Haemolytic assay

Serial of two fold dilution of the toxin (100 µl) was made in normal saline (pH 7.2) starting from the first well to last well with proper mixing. An equal volume of 1% RBC suspension (1 ml of RBC pack mixed in 99 µl of 0.9% saline) was added to each well. The plate was gently shaken and allowed to stand for two hours at room temperature and the results were documented. Appropriate controls (positive and negative) were also included in the titer plate. The reciprocal of the highest dilution of the toxin showing haemolytic activity was taken as one haemolytic unit (HU). The formation of a compact button at the bottom of the well indicated the negative haemolytic activity.

Toxicity assay and determination of LD_{50}

The lethal and paralytic activities were studied in the seashore crab *Ocypode macrocera* (Fig. 1 and 2). The crab weighing 5 ± 2g was collected from the mouth region of Vellar estuary. A crude toxin of *C. diffusa* was injected at the junction of chelate leg. A group of

Fig. 1. Injection of crude toxin

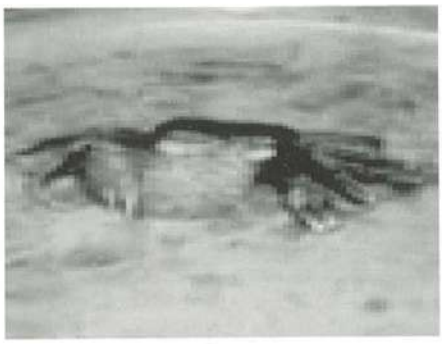

Fig . 2. Foaming of crab

five animals were taken with five deferent doses such as 0, 0.25, 0.50, 0.75, and 1.0 ml of crude toxin. The effect of these concentrations on the crab and symptoms such as colour change, foaming, paralytic effect and restlessness were observed.

Antibacterial susceptibility assay

Antibacterial activity was determined against ten human pathogenic bacteria *Staphylococcus aureus, Salmonella typhii, Salmonella paratyphii, Klebsiella oxytoca, Pseudomonas aeuruginosa, Escherichia coli, Proteus mirabilis, Lactobacillus vulgaris, Vibrio cholerae* and *Klebsiella pneumoniae* by the well diffusion technique in Petri dishes (Kelman *et al.*, 2001). Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms on the surface of Muller Hinton agar plates. Then 20µl of the extract was pipetted out and added inside of the well, the solvent was allowed to evaporate, and the well was placed on the surface of the inoculated agar. In each plate one cup was used for antibiotic Chloramphenicol (100mg/ml) as a control. The test plates were incubated for 24 hrs at 37 °C; solvent controls were performed in each case. Areas of bacterial growth inhibition were observed around the wells. The diameter (mm) of the growth inhibition halos caused by the methanolic extracts of marine sponge was examined. All the assays were carried out in duplicate.

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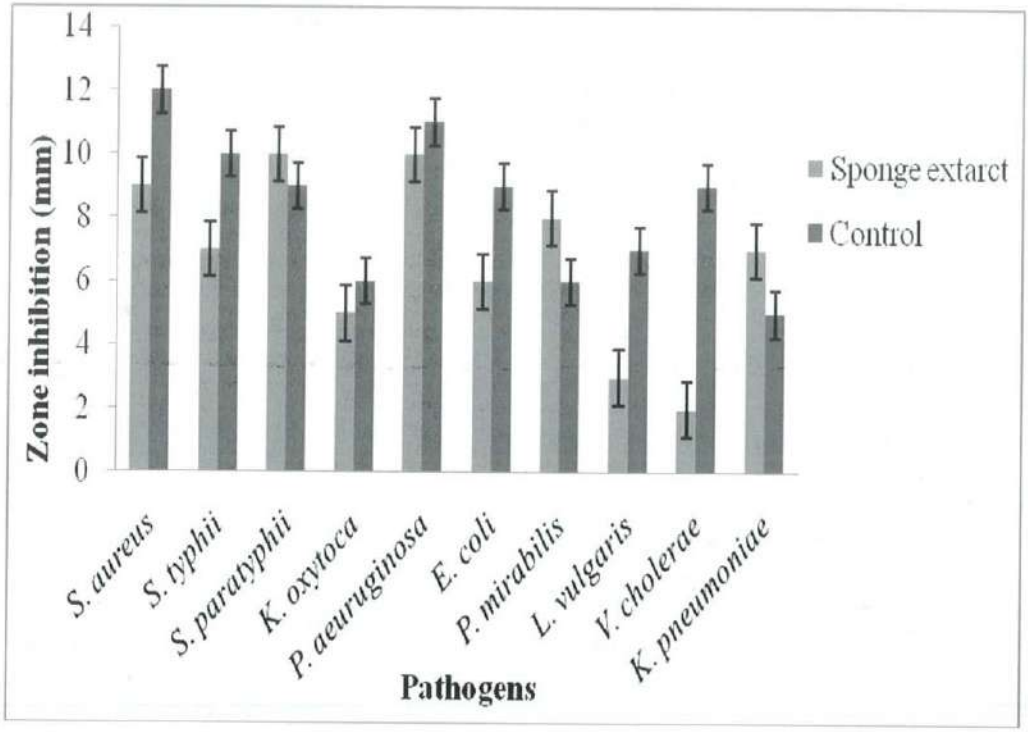

Fig. 3. Antibacterial activity of sponge *Callyspongia diffusa* extract against human pathogenic bacteria.

Table 1. Toxicity assay and determination of LD_{50}.

S.No	Concentration (5mg/ml)	No of animals	Observation				
			Death time (30min)	Colour change	Foaming	Paralytic effect	Restlessness
1	0.25	10	Nil	+	-	+	+
2	0.5	10	Nil	+	-	+	-
3	0.75	10	10	+	+	+	-
4	1	10	10	+	+	-	-

RESULTS AND DISCUSSION

The haemolytic activity test showed very good hemolytic effect in the first wells of goat blood and two wells in chicken blood with the haemolytic effect of 32 HU/g and 16 HU/g respectively. In crab the minimum lethal dose of the toxin extracted from *C. diffusa* was found to be 0.05ml per 5 ± 2 g crab *O. macrocera*. The result of the further observation made on the colour change of the crab, foaming, paralytic effect and restlessness of the crabs is presented in the Table 1. The antibacterial activity of the methanol extract of *C. diffusa* was found effective against ten bacterial pathogens. Maximum inhibition of growth was found 10 mm against *P. aeuruginosa* and *S. paratyphii*. While the minimum of 2.0 and 3 mm was recorded against *V. cholerae* and *L. vulgaris* (Fig 3).

The methanol extract of *C. diffusa* induced pronounced haemolysis on chicken and goat erythrocytes. Fusetani *et al.* (1981) reported that the sterol derivatives from halichondriidie sponges possed good haemolytic activity. Mebs *et al.* (1985) demonstrated the aqueous extracts from sponge species showed haemolytic, hemagglutinating, cytotoxic, antimicrobial, anticholesterase and lethal activities. Stempien (1970) also reported the haemolytic activity from halitoxin. The present study revealed screening of organic extracts from marine sponge is a common approach to identify compounds of biomedical importance.

The antibacterial activity assay revealed that the sponge extract showed highest inhibition of growth against *P. aeuruginosa* and *S. paratyphii*. Research has indicated that the secondary metabolites of sponges play an important role in their defense against infectious microorganisms (Proksch, 1994). A large number of compounds exhibiting antimicrobial activity

with potential biomedical application have been isolated from marine sponges (Laport, 2009). The marine sponges *Acanthella ramose* and *A. cavernosa* from the Bay of Bengal were active against the virulent fish pathogens *A. hydrophila, Edwardsiella tarda, P. Aeruginosa, P. fluorescens* and *V. alginolyticus* (Choudhury *et al.*, 2003). The sponge *H. exigua* is a rich source for several bis-1-oxaquinolizidine alkaloids, exhibiting diverse biological properties such as cytotoxic, antifungal, antimalarial, antituberculosis and anti-rat brain nitric oxide synthase activities (Orabi *et al.*, 2002; Liu *et al.*, 2004).

CONCLUSION

Biological activity of the extract of *C. diffusa* collected from the Mandapam coast was assessed. The results indicated selective biological activity such as haemolytic activity against chicken and goat blood, neurotoxic activity against seashore crab and antimicrobial activity against some selected pathogens, thus showing that Mandapam sponge provide an important source of biologically active secondary metabolites.

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