



Short Communication

Antibacterial and antifungal properties of southeast Indian coastal sponges

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Abstract

Seven species of sponges collected from southeast coast India were tested for antibacterial and antifungal properties. The alcoholic extracts were obtained individually and their sub-fractions were prepared by counter current solvent extraction of the alcoholic extracts to test their bioactivity. Semi-pure fractions were obtained by silica gel column chromatography. The non-polar and medium polar fractions of three sponges viz. *Cervicornia* sp., *Callyspongia fibrosa* and *Acanthella elongata* were found to be active against the tested fish pathogens, *Vibrio alginolyticus*, *V. fischerii*, *Micrococcus* sp. and a fungal strain, *Rhizoctonia solani*, showing the potential of Gulf of Mannar - Palk Bay sponges to have bioactive properties.

Keywords: Sponges, bioactive compounds, antibacterial compounds, marine natural products

Introduction

Sponges are considered as storehouse of extraneous organisms like bacteria, cyanobacteria and epibionts as their symbionts (Lee *et al.*, 2001). Some of them are endosymbionts occupying intracellular space accounting for copious amount of biomass of the respective host. The symbionts produce chemicals for their mutual benefits. There are increased evidence that compounds found in sponges are biosynthesized by these microbes. However, this is not confirmed so far. Some of the compounds are indeed used in chemotaxonomical studies as the case of verongid sponges (Cimino *et al.*, 1975) with bromotyrosin derivatives.

Some of the prominent compounds of sponges are ilimaquinone (Luibrand *et al.*, 1979) avarol, mero sesquiterpenes (Crispino *et al.*, 1989), bengamides (Zia *et al.*, 2001), long chain fatty acid derivatives (Proksch *et al.*, 2003), manzamine alkaloids which are antimalarial and antituberculosis (Rao *et al.*, 2004) and halichondrins polyether macrolides which are antitumor (Hickford *et al.*, 2009). Sponges

continue to contribute a variety of new compounds and are the commodity of major attention (Blunt *et al.*, 2009). Gulf of Mannar-Palk Bay from Point Calimer to Kanyakumari is a rich biodiverse marine zone housing more than 275 species of sponges. However systematic chemical exploration of invertebrates including sponges in this area is limited. In our search for bioactive compounds that can control pathogens of some commercially important fishes, we explored seven sponges from southeast coast of India for antibacterial compounds/fractions.

From Palk Bay two samples namely *Cervicornia* sp. (Order: Hadromerida, Family: Clionidae) and *Hippospongia* sp. (Order: Dictyoceratida, Family: Spongiidae), and from Kanyakumari coast five samples namely *Callyspongia fibrosa* (Order: Haplosclerida, Family: Callyspongiidae), *Hyattella cribriiformis* (Order: Dictyoceratida, Family: Spongiidae), *Callyspongia* sp., *Clathria vulpina* (Order: Poecilosclerida, Family: Microcionidae) and *Acanthella elongata* (Order: Halichondrida, Family: Axinellidae) were collected. All sponge samples were identified by the taxonomist, Dr. P. A. Thomas.

Material and Methods

The sponges (weight: 300-900 g) were collected from the intertidal zone and as a by-catch from the trawlers. The fresh specimens were preserved in ethanol immediately after collection and transported to the laboratory for analysis. The sponges were individually exhaustively extracted with ethanol to get alcoholic extract. The alcoholic extract was used as such or sub-fractionated using petroleum ether, carbon tetrachloride, dichloromethane, *n*-butanol or ethyl acetate to test antibiotic activity against fish pathogens and the fungus. Sub-fractions were obtained by counter current separation using appropriate solvent from the alcoholic extract. Purification of the sub-fraction was done by silica gel column chromatography with petroleum ether elution to get semi pure fractions. The semipure fractions of two sponges, *Cervicornia* spp. and *Hippospongia* spp. of Palk Bay were taken for the study (Rajendran, 2005). Each sub-fraction of Kanyakumari coast sponges was also tested for biological activity against fish pathogens, *V. alginolyticus*, *V. fischerii* and *Micrococcus* spp. and a fungal strain *Rhizoctonia solani*. The filamentous fungus *Rhizoctonia solani* obtained from MTCC, IMTEC, Chandigarh was used to test the antifungal activity of sponge extracts / fractions.

Antibacterial testing by disc assay method:

Sterile paper discs (6 mm diameter, Himedia, Mumbai) were impregnated with three drops of respective extracts dissolved in appropriate organic solvent and kept for evaporation of the solvent under aseptic conditions. The discs were then placed in nutrient agar plates inoculated with broth culture (in the case of *V. alginolyticus*) or spore suspension (in the case of fungus *R. solani*) of the respective pathogen along with control discs soaked in the organic solvent, after evaporation of the solvent. The plates were then incubated at ambient temperature for 48 h. The zone of inhibition of bacterial growth around the disc was then measured. The assay was scored positive if the zone of inhibition was < 2 mm, double positive if ≥ 2 mm, negative if there was no inhibition of microbial growth.

Results and Discussion

The consolidated results of activity tested against pathogens and fungus are summarised in Table 1. The gas chromatography–mass spectroscopy analysis of the carbon tetra chloride fraction of *Cervicornia* spp. revealed the presence of long chain saturated and unsaturated fatty acid methyl ester of chain length C-14 to C-24. It had inhibition against *V. alginolyticus* as commonly observed among the unsaturated fatty acids, whereas the semipure fraction obtained from

Table 1. Antibacterial and antifungal activity exhibited by sponge extracts

Sponge species	Extract tested	Organisms tested				
		<i>V. alginolyticus</i> (Strain I, E-41)	<i>V. alginolyticus</i> (Strain II, Lob-5B)	<i>V. fischerii</i>	<i>Micrococcus</i> spp.	<i>R. solani</i>
<i>Cervicornia</i> spp.	Carbon tetrachloride	(+)	(+)	--	--	--
<i>Hippospongia</i> spp.	Petroleum ether	(-)	(-)	--	--	--
<i>Callyspongia fibrosa</i>	Hexane	(-)	--	--	--	(-)
	Carbon tetrachloride	(-)	--	--	--	(-)
	Dichloro methane	--	(+)	--	--	(++)
	<i>n</i> -butanol	(+)	(+)	--	--	--
<i>Hyattella cribriformis</i>	Ethanol	(-)	(-)	--	--	--
<i>Callyspongia</i> spp.	Ethanol	(-)	(-)	--	--	--
<i>Clathria vulpina</i>	Ethanol	(-)	(-)	--	--	--
<i>Acanthella elongata</i>	Ethanol	--	--	(+)	(+)	--
	Petroleum ether	--	--	(++)	--	--
	Dichloro methane	--	--	--	(++)	--

Signs in parenthesis note the tested activity; (+) - if zone is < 2mm; (++) - if zone is ≥ 2 mm; (-) - no inhibition

the Petroleum ether solubles of the alcoholic extract of *Hippospongia* sp. did not have activity. The Petroleum ether fraction of *Hippospongia* sp. was found to contain steroidal compounds like cholesterol, τ - sitosterol and their derivatives.

Among the fractions of Kanyakumari coast sponges tested, the dichloro methane fraction of *C. fibrosa* showed antibacterial activity against *V. alginolyticus*, and also antifungal activity against *R. solani*. The alcoholic extracts of the sponges, *H. cribriformis*, *Callyspongia* sp. and *C. vulpina* did not show any activity against two strains of *V. alginolyticus*.

The alcoholic extract of *A. elongata* was having inhibition on bioluminescent bacteria *Vibrio fischerii* and a gram positive bacterium *Micrococcus* sp. whereas the dichloro methane soluble fraction was found to be active against *V. alginolyticus* (zone of inhibition – 7 mm diameter) and *R. solani* (zone of inhibition – 16 mm diameter).

Thus, among the sponges taken for the study, it was found that dichloro methane fractions of *C. fibrosa* and *A. elongata* showed anti bacterial activity against *V. alginolyticus* and antifungal activity against *R. solani* indicating the presence of active components in medium polar dichloro methane fraction.

The active fractions of the alcoholic extract of sponges of both Palk Bay and Kanyakumari coasts have the potential for active compounds which may be effective for fish disease control.

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