

Antiamoebic Activity of Marine Sponge *Spongia officinalis* var. *ceylonensis* Dendy

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Abstract

Human amoebiasis due to infection by *Entamoeba histolytica* is mainly associated with morbidity thus affecting the quality of life and pace of developmental activities of countries with warm climatic conditions. A consistently high global incidence of this disease has been reported. In an endeavor to identify novel molecules with potent antiamoebic activity and lower side-effect, the present study was designed to investigate antiamoebic activity in *Spongia officinalis* extracts/fractions followed by isolation and characterization of bioactive molecules. The methanol extract of the *S. officinalis* showed promising *in vitro* and *in vivo* antiamoebic activity. On further fractionation, the activity was localized in the *n*-butanol soluble fraction from which the major purified fraction was identified as a mixture of alkaloids (xestospongins and araguspongins) by LCMS studies of the *n*-butanol soluble fraction.

Key words: Antiamoebic activity, alkaloids, *in vitro* and *in vivo*, marine sponge, *Spongia officinalis*

Introduction

Human amoebiasis due to *Entamoeba histolytica* infection is mainly associated with morbidity thus affecting the quality of life and pace of developmental activities of countries with warm climatic conditions. A consistently high global incidence of this disease has been reported from surveys carried out at different intervals of time (Thomas *et al.*, 2010). This disease also possesses a challenge to our national medical and health programmes. A number of therapeutic agents possessing potent *in vitro* action against trophozoites of *E. histolytica* have been used to combat this disease. So far, these have been found to be too toxic or providing only symptomatic relief. Leads to obtain novel molecules with antiamoebic activity have been obtained from natural products, either terrestrial plants or marine organisms. Marine sponges are incredible source of novel pharmacologically active compounds (Thomas *et al.*, 2010) which have earlier shown potent efficacy against various diseases. They are known to possess diverse pharmacological activity in several diseases such as cancer, neurodegeneration, type-2-diabetes, fungal and microbial infections (Bhosale *et al.*, 1999; Dembitsky *et al.*, 2005; Rao *et al.*, 2006; Coello *et al.*, 2009; Guzmán *et al.*, 2011; Takei *et al.*, 2010). These

biological activities have been attributed to the presence of novel sterols, metabolites including steroids, terpenoids, alkaloids, cyclic peptides and unsaturated fatty acids (Litchfield *et al.*, 1976; Findlay and Patil, 1985; Elenkov *et al.*, 1999). With a view to explore the possibilities of finding new molecules with proven therapeutic efficacy for human use, a programme is operational at the Central Drug Research Institute, Lucknow, India for screening of extracts of marine organisms for a wide range of biological activities. The programme consists of collection, identification and extraction of marine flora and fauna along the Indian coasts for biological screening. *Spongia officinalis* was selected for detailed chemical and biological investigations with a view to isolate bioactive compounds.

S. officinalis var. *ceylonensis* Dendy belongs to the Phylum Porifera, Class Demospongiae and Family Spongiidae. Sponges are utilized as therapeutic agents. A literature search revealed that few compounds have been reported from *S. officinalis*, including alkaloids and steroids (Lin *et al.*, 2003; Lin *et al.*, 2001; Thale *et al.*, 2002; Orabi *et al.*, 2002; Edrada *et al.*, 2002; Williams *et al.*, 2002; Bohlin *et al.*, 1980).

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Materials and Methods

Collection of marine animals: *S. officinalis* var. *ceylonensis* collected from the Ramnad of the Tamil Nadu coast of India in the month of November. A specimen has been preserved in the herbarium of the Botany Division of the Institute with the specimen number 412.

Extraction, fractionation, and isolation of pure compounds: The freshly collected marine sponges *S. officinalis* (2.0 kg) were thoroughly washed with distilled water to remove extraneous materials and filled in steel containers and soaked in methanol. This was then transported to the laboratory of Central Drug Research Institute, Lucknow, India. The methanol extract was drained off and the animals were chopped into small pieces and filled in a glass percolator soaked in methanol and left for 24 h at room temperature. The process of extraction was repeated four times and the combined percolates were concentrated under reduced pressure below 50 °C in a rotavapor to provide a green viscous mass. It was further dried under high vacuum for two hours to remove last traces of the solvent (weight 40.0 g). A portion of the crude extract (25.0 g) was fractionated into four fractions by macerating with *n*-hexane, chloroform, and *n*-butanol successively. The *n*-hexane (0.45 g), chloroform (2.25 g), and *n*-butanol (4.25 g), materials along with *n*-butanol insoluble (18.0 g) fractions (Fig.1) were evaluated for anti-amoebic activity by *in vitro* model. The maximum anti-amoebic activity was localized in the *n*-butanol soluble fraction. It was analyzed by LCMS and xestospongins and araguspongins were identified as major compounds (Venkateshwarlu *et al.*, 1994). Separation and purification of the pure compounds is in progress and will be reported separately.

Test models and methodology for anti-amoebic activity

In-vitro model: Axenic culture of *E. histolytica* (200: NIH) maintained TYI-S-33 medium (Diamond *et al.*, 1978) has been used for *in-vitro* screening. Xenic culture 2771 isolated from an acute case and maintained in Robinson's medium (Robinson, 1968) was used to produce experimental caecal amoebiasis in rats.

Evaluation of in vitro amoebicidal activity: The stock solution of the test agent is prepared by adding small quantity of DMSO and required amount of water. Further serial double dilutions were prepared using triple glass distilled water. Amoebic inoculum (0.1 ml) containing

approximately 2000 trophozoites were added to the cavities of shallow cavity slides to which the test sample (0.1 ml) in its required dilution was added. Each cavity was then sealed with cover slip. The slides were kept in the moist chamber at 37 °C. Observations were made at 24 and 48 hrs intervals. The activity of the test agent at the particular dilution was related with cent percent mortality. Metronidazole was the standard compound used. Duplicate sets were kept for each dilution (Das, 1975)

Anti-amoebic *in-vivo* test model

Experimental production of caecal amoebiasis of rats: Rats were fed on autoclaved rice diet for seven days prior to infection. The caecal contents of these rats attained a pH of 5.5 to 7.0 without the occurrence of free ammonia which is toxic to these amoebae (Prasad and Bansal, 1983; Leitch, 1988) thus aiding in the consistent production of caecal infection. Rats under ether anesthesia were inoculated intracaecally with 0.2 to 0.3 ml of amoebic inoculum containing 10×10^4 trophozoites of *E. histolytica* and the abdominal lesion sutured. After 48 hr the infected rats were ready for therapeutic evaluation of test agents as trophozoites of *E. histolytica* were visible microscopically in the contents and scrapings of the caecal wall. The animals were divided into two groups. One group was given oral administration of the drug, while the other group served as control group.

Treatment schedule: The test material was suspended in gum acacia suspension in distilled water. The rats were orally administered with the test agent at (900) mg/kg b.w. with the help of a feeding needle once daily for five consecutive days. The rats were sacrificed 48 hr. after the last dose of test material with an overdose of ether anesthesia and the caecum examined for trophozoites of *E. histolytica*. The reported method of Neal (1951) was used to evaluate the degree of infection.

Results and Discussion

The effects of *S. officinalis* extracts on trophozoites of *E. histolytica in vitro* and against caecal amoebiasis of rats have been summarized in Table 1. *In vitro* efficacy was recorded for all the test samples. The *in vivo* therapeutic efficacy of the crude extracts presented interesting results. It was observed that the methanol extract when administered at a dose of 250 mg /kg body weight for five days effected 100% cures. The *n*-butanol and aqueous

fractions of the same extract exhibited high efficacy with 100% cures at 100mg/kg dose b.w. (Table 1).

It is not uncommon that marine organisms possess activity against pathogenic bacteria, fungus and protozoa. The terpenoids isolated from *Pseudoplenauria wagaenari* possesses antiamebic activity *in vitro* model. Lobane diterpene derivatives of this organism were active against

phytopathogenic fungus, *Cladosporium cucumerinum*, gram positive bacteria, *Bacillus subtilis*, and yeast, *Saccharomyces cerevisiae* (Edrada *et al.*, 1998). Similar derivatives have also been isolated from other marine organisms (Shin and Fenical 1991).

Table 1. Results of antiamebic activity of *Spongia officinalis* var. *ceylonensis* Dendy against *E. histolytica* in *in-vitro* and *in-vivo* models.

Name of the exts./frs.	Antiamoebic activity against <i>E. histolytica</i>		
	<i>In- vitro</i> MIC ($\mu\text{g/ml}$)	<i>In- vivo</i>	
		Dose	mg/kg (for 5 days)
Methanol ext.	31.25	250	100
50% MeOH-CHCl ₃ extract	125	250	20
Hexane sol. fraction from methanol extract	125	100	25
Chloroform soluble fraction from the methanol ext	125	100	30
<i>n</i> -Butanol soluble fraction of the methanol ext	31.25	100	100
Aqueous fraction of the methanol extract	31.25	100	100
Metronidazole (Standard)	10	50	60
		100	100

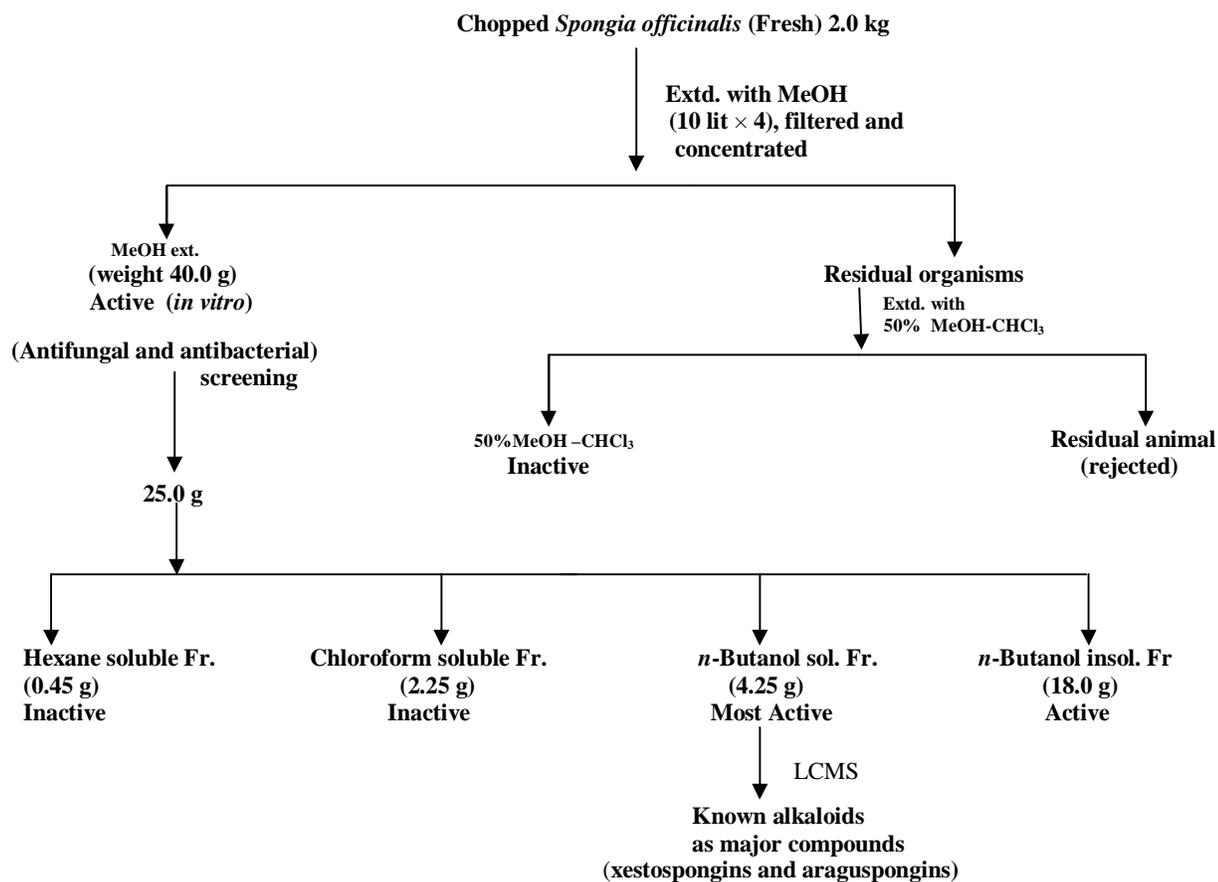


Figure 1. Isolation of bioactive compounds from *S. officinalis*

In view of the results presented, it is evident that marine organisms can provide leads for antiameobic agents in future. Thus, the ocean with its innumerable biota offers a challenge to both chemists and biologists alike as it is a large reservoir of novel chemical entities with therapeutic potential for human use.

The results assumed significance when viewed regarding the condition of the caecal wall. The caecum of rats receiving the crude extract and the *n*-butanol soluble fraction appeared normal with thin caecal wall comparable to the rats treated with the standard drug metronidazole (100mg/kg body weight). However, the caecal contents of the rats treated with the test agents although being normal was slightly less formed as compared to the metronidazole treated rats. The results become still more interesting when the caecum of the treated rats were compared with the untreated rat caecum which is shapeless with ulcers on the walls and with mucous and very little faecal matter as contents.

It is apparent from the results that *S. officinalis* possessed significant amoebicidal activity against *E. histolytica*. In the present study, the active fractions possessed 100% activity at 100mg/kg dose for 7 days has been identified. Development of these fractions into a viable drug requires further isolation of active molecules from these fractions and structural characterization of the active compounds are being pursued in our laboratory and will be reported later. This validates the promise held by the ocean as a source of therapeutic agents against human ailments.

Declaration of interests

All the authors have no conflict.

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