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In vitro and *in vivo* antiamoebic activity of the *Heritiera littoralis* Dryand against *Entamoeba histolytica*

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ABSTRACT

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. 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 *Heritiera littoralis* extracts. The aqueous ethanol and aqueous extracts of the *Heritiera littoralis* showed promising *in vitro* and *in vivo* antiamoebic activity (MIC 31.25 and At 250 mg/Kg dose 90% protection). Further work on pure molecules from this plant is in progress to get an antiamoebic lead molecule.

KEYWORDS

Antiamoebic activity;
Heritiera littoralis;
Entamoeba histolytica;
in vivo.

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[1]. This disease also poses 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[1] which have earlier shown potent efficacy against various diseases. Marine sponges are known to possess diverse pharmacological activity in several diseases such as cancer, neurodegeneration, type-2-diabetes, fungal and microbial infections[2-7]. These biological activities have been attributed to the presence of novel sterols, metabolites including steroids, terpenoids, alkaloids, cyclic peptides and unsaturated fatty acids[8-10]. 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.

Heritiera littoralis was selected for detailed chemical and biological investigations with a view to isolate bioactive compounds.

Heritiera littoralis Dryand belongs to the family Sterculiaceae. is an evergreen mangrove tree, up to 25 m in height and with a buttressed trunk up to 60 cm in diameter. The bark is fissured, dark or gray. Leaves are 10–20 cm long, and they have a green upper surface and a silvery-white lower surface. The tree has numerous small bell-shaped, yellowish-green flowers. The fruits are hard and shining, 4–8 cm long[11].

H. littoralis seed extracts are traditionally used to treat diarrhoea and dysentery[12]. The stems and leaves have also been used against diarrhoea and dysentery. In addition, they have been used to control mosquitos and as a piscicide[13,14,15]. The sap is reported to be a fish poison and arrowhead and spearhead poison[12,16]. The seeds and leaves are, however, regarded as edible in the Andaman and Nicobar islands[17]. The tree is used as tooth brushes and chew sticks. The wood is also valuable for its timber[18]. A literature search revealed that few compounds have been reported from *H. littoralis* are isofraxidin, friedelin, betulinic acid, β-sitosterol, stigmasterol, sitost-4-en-3-one, ergosterol peroxide, physcion[12] quercitrin, quercetin, kaempferol-3-O-(63 -O-E-p-coumaroyl)-β-d-glucopyranoside, kaempferol, kaempferitrin, myricetin, eriodictyol, afzelin, astragalin, tribuloside, catechin[19] and many more others. The bioactivity reported antidiarrhoeal use of H. littoralis, Aqueous leaf and stem extracts of the plant have shown antibacterial activity against *Salmonella paratyphoid*, while the ethanol extract was inactive. Some other bacteria, e.g., *S. aureus and P. aeruginosa* were also inhibited. Several triterpenoids and steroids have shown anti-inflammatory activity determined as NO inhibitory effect and anti-PGE_2 activity, with ergosterol peroxide being the most active substance[20]. Since there are no report on the antiamoebic property of this plant we have selected this plant for initial antiamoebic screening.

MATERIALS AND METHODS

Collection of marine animals

Aerial parts of the *H. littoralis* mangrove were collected from South Andaman Coast in the month of January 1998. Specimen sample (voucher specimen number, 325) has been preserved in the herbarium of the Botany Division, Central Drug Research Institute, Lucknow, India.

Extraction procedure

Air dried powdered aerial parts of the *H.littoralis* (250 g) were extracted with 95% ethanol (5 x5.0 lit) and the combined extracts were filtered, concentrated under reduced pressure below 50^0 C to minimum volume of 1.0 lit. It was further dried in hot air vacuum oven at 45^0 C to green powder (yield 15%). Further 250g of the plant material was extracted with 50% aqueous ethanol as above and the green powder (yield 22%). The plant material (250 g) was also extracted with triple distilled water and method was repeated as described above to get the extract, this was finally dried in a lyophillizer to get powder (yield 25%). These three different extracts were used to screen antiamoebic activity.

Test models and methodology for antiamoebic activity

(a) *In-vitro* model

Axenic culture of *E. histoyitica* (200: NIH) maintained TYI-S-33 medium[21] has been used for *in-vitro* screening. Xenic culture 2771 isolated from an acute case and maintained in Robinson's medium[22] was used to produce experimental caecal amoebiasis in rats.

Evaluation of in-vitro amoebicidal activity

The stock solution of the test agent was 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 was added to the cavities of shallow cavity slides to which the test sample (0.1 ml) in its required dilution is added. Each cavity was then sealed with cover slip. The slides were kept in the moist chamber at 37°C. Observations were taken at 24 and 48 hrs intervals. The activity of the test agent at the particular dilution was related with percentage of mortality. Metronidazole was the standard compound used. Duplicate sets were kept for each dilution[23].

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TABLE 1 : Results of antiamoebic activity of *H.littoralis* against *E.histolytica* in *in-vitro* and *in-vivo* models

Name of the extracts	Antiamoebic activity against *E.histolytica*		
	In-vitro MIC (μg/ml)	*In- vivo*	
		Dose mg/kg (days)	% inhibition
Ethanol extract.	62.5	250 (7)	60
50%EtOH-extract.	31.25	250 (7)	90
Aqueous extract.	31.25	250(7)	90
Metronidazole (Standard)	10	50 (7) 100 (7)	60 100

Experimental production of caecal amoebiasis of rats

(a) Antiamoebic *in-vivo* test model

Rats were fed on autoclaved rice diet for seven days prior infection. The caecal contents of these rats attain a pH of 5.5 to 7.0 without the occurrence of free ammonia which is toxic to these amoebae[24,25] 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 x 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.*hist*olytica* 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 administered orally the test agent at 250 mg/kg 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[26] was used to evaluate the degree of infection.

RESULTS AND DISCUSSION

The effect of *H.littoralis extracts* on trophozoites of *E.histolytica in vitro* and against caecal amoebiasis of rats is described in TABLE 1. *In vitro* efficacy was recorded for all the test samples. The *in vivo* therapeutic efficacy of the all extracts presented interesting results. It was observed that the 50% aqueous ethanol and aqueous extracts when administered at a dose of 250 mg /kg body weight for five days effected 90% cures (TABLE 1).

It is not uncommon that marine organisms possess activity against pathogenic bacteria, fungus and protozoa. The terpenoids isolated from *Pseudoplenauria wagenaari* posses antiamoebic 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 cerevisia*[27] Similar derivatives have also been isolated from other marine organisms[28].

In view of the results presented it is evident that marine organisms can provide leads for antiamoebic 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 50% aqueous ethanol crude extract and aqueous extract 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 are 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 *H.littoralis* possesses significant amoebicidal activity against *E.*

histolytica. In the present study, the active extracts possess 90% activity at 250mg/Kg dose for 7 days has been identified.

CONCLUSION

Development of these extracts into a viable drug requires further isolation of active molecules from these extracts and structural modification of the active compounds is 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.

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DECLARATION OF INTERESTS

All the authors have no conflict.

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