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Antiamoebic potential of gedunin from *Xylocarpus granatum* Koen

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ABSTRACT

Objective: A number of therapeutic agents possessing potent *in vitro* action against trophozoites of *E. histolytica* have been used to combat this disease but so far, these have been found to be too toxic or providing only symptomatic relief. Therefore it was planned to search the molecules from natural sources.

Material and methods: Fruits of the X*ylocarpus granatum* mangrove were selected for the study and were collected from South Andaman Coast. Ethanol extract as well as four fractions (hexane, chloroform, n-butanol soluble and n-butanol insoluble fractions) were prepared and active principle gedunin was isolated from the active fraction.

Results and discussion: The ethanol extract showed MIC 125 µg /ml in our *in-vitro* studies, and *in vivo* showed 80% inhibition of trophozoites at the dose of 900 mg/kg body weight against *Entamoeba histolytica*. Only chloroform soluble fraction showed 80% inhibition of trophozoites at 900 mg/kg dose. The active principle from active fraction showed 100%inhibition at 300mg/kg dose.

Conclusion: Structural modification of the Gedunin is required for further enhace the antiamoebic activity. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Anti-amoebic activity;
Gedunin;
Xylocarpus granatum;
Against *entamoeba histolytica*.

INTRODUCTION

Human amoebiasis is due to *Entamoeba histolytica* infection. It 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[1,2]. This disease also poses a challenge to our national health programme. 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 that leads to obtain novel molecules with antiamoebic activity from natural products, either terrestrial plants or marine organisms. The scope of natural products have widened with the inclusion of marine biota. Sponges are known as a rich source of sesquiterpenes and diterpenes and alkaloids.

Drug from marine resources is an area which offers an unprecedented opportunity for their pharmacological exploration and hence has received great attention during recent years for natural product chemistry, a promising new area of study. Secondary metabolites produced in marine organisms could be the source of bioactive substances which may be useful in modeling compounds for drugs[3,4]. Marine microorganisms,

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whose immense genetic and biochemical diversity is only beginning, likely to become a rich source of novel chemical entities for the discovery of more effective drugs.

Xylocarpus granatum Koen. belongs to Natural Order Meliaceae. It is a mangrove and is commonly known as pussur in Hindi language. It is tall tree ranging upto 20 m. with buttressed stem base. Bark is yellowish-white, peeling off as papery flakes. Leaves uni-jugate or bi-jugate, Leaflets obovate, glabrous, entire, rounded at apex, tappering at base; flowers 5-7 mm. across, white with a reddish gland within, in axillary thyrses : Calyx 4- lobed, petals 4, free. Fruits are large as long as 30 - 40 cm. across, globose, septa fragal capsules; splitting tardily into 4 valves. Seeds 10- 15 in number pyramid shaped corky testa. Flowering and fruiting throughout year. In India the species occurs in tidal forests along the East and West coastal areas upto Maharastra and in Andaman Island. Seed paste is used for relief of breast cancer[5,6]. Literature review revealed that only few workers have tried to isolate chemical constituent of this species. Fatty acids, sterols and hydrocarbons were isolated from its leaves[7]. An alkaloid 8- acetyl dihydrochelerythrins from its root bark[8]. 7-α- Acetoxydihydronomillin (Cneorin-G) was isolated from this plant[9,10]. Xylocarpin was isolated from the seed of the plant[11] In another report Xyloccensin - I & J were also isolated from this species[12]. Further in another report, Xyloccensin-K, a new limonoid was also isolated from the seeds[13]. 6-α, 11-β-diacetoxygedunin were isolated from its fruits[14]. All these research papers are of academic interest and no reports are available on bioactivities of isolated compounds. In one of the report Gedunin has been reported as antidiabetic compound[15]. The present communication deals with the amoebicidal activity *Xylocarpus granatum*, a mangrove plant against trophozoites of *E. histolytica* both *in vitro* and against experimental caecal amoebiasis of rats.

MATERIAL AND METHODS

Collection of material

Fruits of the *Xylocarpus granatum* mangrove were collected from South Andaman Coast in the month of January 1999. Specimen sample (voucher specimen number, 332) has been preserved in the herbarium of the Botany Division, Central Drug Research Institute, Lucknow, India.

Extraction/fractionation/isolation procedures

Air dried powdered fruits (1.0Kg) 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 brown powder (yield15%). The brown powder was further fractionated into chloroform soluble (yield 8.5%of the 95%.ethanolextract) and chloroform insoluble fractions by maceration with chloroform. The chloroform fraction on repeated column chromatography over silica gel and final purification by HPLC on reverse phase C_{18} R.P columns using acetonitrile-water 55:45, v/v,flowrate-1.0 ml/ min using UV detector (λ 230 nm) yielded compounds namely Gedunin[16]. Photogedunin[17], Xyloccensin-I[12] and Palmitic acid[18]. The Structures of all compounds have been shown in Figure 1. All these were characterized using IR, NMR, mass, derivatization and comparing the data with those given in literature for these compounds. These were also compared with authentic samples on thin layer plates as well as their spectral data.

Test models and methodology for antiamoebic activity

(a) In-vitro model

Axenic culture of *E. histoyitica* (200: NIH) maintained TYI-S-33 medium[19] has been used for *in-vitro* screening. Xenic culture 2771 isolated from an acute case and maintained in Robinson's medium[20] was used to produce experimental caecal amoebiasis in rats.

(A) 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 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 cent percent mortality. Metronidazole was the standard compound used. Duplicate sets were kept for each dilution[21].

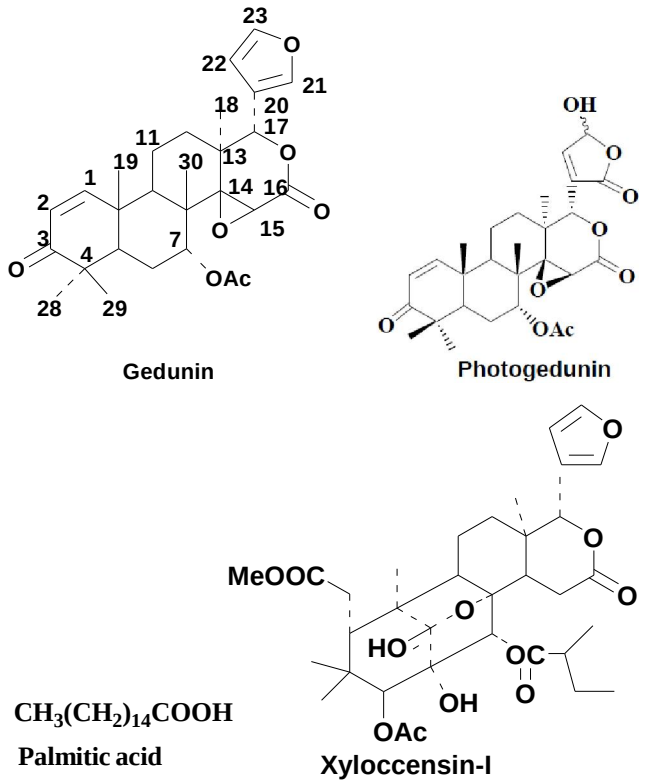

Figure 1 : Structures of the pure compounds isolated and characterized.

(a) Antiamoebic in-vivo test model method

(A) Experimental production of caecal amoebiasis of rats

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[22,23] 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 hours the infected rats were ready for therapeutic evaluation of test agents as trophozoites of *E.histolytica.* These 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.

(B) Treatment schedule

The test material was suspended in gum acacia suspension in distilled water. The rats were administered orally the test agent at 900 mg/kg with the help of a feeding needle once daily for five consecutive days. The rats were sacrificed 48 hours 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 effect of the fruits of the *Xylocarpus granatum* extract 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 including the pure compounds. The *in vivo* therapeutic efficacy of the crude extracts showed that the ethanol extract when administered at a dose of 900 mg /kg body weight for five days effected 80% cures. Chloroform fractions of the same extract exhibited high efficacy with 80% cures at 900 mg/kg dose. Purified compounds Palmitic acid and Gedunin from Chloroform fraction showed promising results executing 80% cures at 300 mg/kg. However, although the caecal wall and contents of all the treated rats were normal some amoebae were observed in the caecal smears. When the time schedule of the rats receiving 300 mg/kg was increased by two days (7 days) a result of 100% was continuously obtained. The entire drug treated rats exhibited ceacum with normal contents comparable to the rats treated with standard drug metronidazole at 100 mg/kg for 5 days.

It is not uncommon that marine organisms possess activity against pathogenic bacteria, fungus and protozoa. Bhosale[24] has reported that terpenoids isolated from *Pseudoplenauria wagenaari* possess antiamoebic activity *in vitro*. Lobane diterpene derivatives of this organism were active against phytopathogenic fungus, *Cladosporium cucumerinum*, gram positive bacteria, *Bacillus subtilis*, and yeast, *Saccharomyces cerevisia*[25]. Similar derivatives have also been isolated from other marine organisms[26]. In view of the results presented it is evident that marine

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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 crude extract and the n-butanol fraction appeared normal with thin caecal wall comparable to the rats treated with the standard drug metronidazole (100 mg/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.

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

Name of the exts./frs/compds	Antiamoebic activity against *E.histolytica*		
		In – vivo	
	In – vitro MIC µg/ml	Dose mg/kg (days)	% inhibition
Ethanol extract	125	900 (5)	80
		500 (5)	40
Hexane sol. Fraction from ethanol extract.	125	900 (5)	30
Chloroform soluble fraction from the methanol ext.	62.5	900 (5)	80
n-Butanol soluble fraction of the methanol ext.	125	900 (5)	20
Aqueous fraction of the methanol extract.	250	-	-
Gedunin	62.5	300 (5)	80
		300 (7)	100
Palmitic acid	125	300(5)	50
Metronidazole (Standard)	8	50 (5)	60
		100 (5)	100

It is apparent from the results that *Xylocarpus granatum* possesses significant amoebicidal activity against *E. histolytica*. In the present study, the active constituent possessing 100% activity at 300 mg/kg dose for 7 days has been identified. Development of this compound into a viable drug requires further reduction of the dose hence; structural modification of the active compound (Gedunin) 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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