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In vitro and *in vivo* antiamoebic potential of *Phoenix paludosa* Roxb. leaves against *Entaamoeba histolytica*

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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 *Phoenix paludosa* extracts. The ethanol extract of the *P. paludosa* showed promising *in vitro* and *in vivo* antiamoebic activity.

KEYWORDS

Antiamoebic activity;
In vitro and *in vivo*;
Mangrove plant
P. paludosa.

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 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[1] 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[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. *P. paludosa* was selected for detailed chemical and biological investigations.

Phoenix paludosa Roxb. (paludosa, Latin, swampy), also called Mangrove Date Palm, is a spe-

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cies of flowering plant in the palm family, indigenous to coastal regions of India, Bangladesh, Myanmar, Thailand, Cambodia, Sumatra, Vietnam and peninsular Malaysia. The trees grow in clusters, to 5 m high, usually forming dense thickets. The leaves are 2 to 3 m long and recurved. The genus *Phoenix* is reported to have diuretic, analgesic[11], ameliorative[12], antioxidant and antimutagenic activities[13,14]. Previous phytochemical dione and vitexin[15].

MATERIALS AND METHODS

Collection of plant material

P. paludosa leaves were collected from the Andaman coast of India. A specimen has been preserved in the herbarium of the Botany Division of the Institute with the specimen number 327.

Extraction procedure

The freshly collected leaves of *P. paludosa* were shade dried and powdered. The powdered leaves (1.0 Kg.) were soaked in ethanol (4x5 lit) and the combined extract was filtered and concentrated under reduced pressure below 50^0 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 26.0 g). The remaining plant material was further extracted with 50% aqueous ethanol 4x5.0 lit.) These two extracts were evaluated for antiamoebic activity by *in vitro* model. The 50% aqueous ethanol extract was found more active as compared to pure ethanol extract. Both of these were tested in vivo model.

TEST MODELS AND METHODOLOGY FOR ANTIAMOEBIC TESTING

In-vitro model

Axenic culture of E. histoyitica (200: NIH) maintained TYI-S-33 medium[16] has been used for *in-vitro* screening. Xenic culture 2771 isolated from an acute case and maintained in Robinson's medium[17] 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 ^{o}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[18].

Evaluation of in-vivo amoebicidal activity

(a) 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[19,20], 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 104 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[21] was used to evaluate the degree of infection.

RESULTS AND DISCUSSION

The effects of *P. paludosa* extracts on trophozoites of *E. histolytica in vitro* and against caecal amoebiasis of rats have been summarized in TABLE 1. *In*

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

Name of the extract	*In vitro* MIC (µg/ml)	*In vivo*	
		Dose mg/kg (for 5 days)	% Inhibition
Ethanol extract	62.5	250	60
50% Aqueous ethanol extract	31.5	250	90
Metronidazole (standard)	10	50	60
		100	100

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 ethanol extract when administered at a dose of 250 mg /kg body weight for five days effected 60% cures. The 50% aqueous extract of the same exhibited high efficacy with 90% cures at 250 mg/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 wagenaari* possesses antiamoebic activity in vitro model. Lobane diterpene derivatives of this organism were active against phytopathogenic fungus, *ladosporium cucumerinum*, gram positive bacteria, *Bacillus subtilis*, and yeast, *Saccharomyces cerevisiae*[22]. Similar derivatives have also been isolated from other marine organisms[23]. Further work is required on this mangrove to characterize lead molecule to develop it as antiamoebic agent.

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