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In-vitro Trematocidal Effect of *Jatropha gossypifolia* Root Against *Paramphistomum explanatum.*

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ABSTRACT

Jatropha gossypifolia under the family Euphorbiaceae is well known as Lal bherenda. It has versatile traditional uses including efficacy against worm. An attempt was taken to establish its traditional use for its efficacy against trematode (helminth), *Paramphistomum explanatum*. Trematodes were incubated with petroleum ether extract (60-80˚C) extract (PEJG) at concentration of 1, 2, 5, 10, 25 mg/ml along with albendazole (10 mg/ml) as standard at room temperature. Efficacy of the extract was judged by its effect on paralytic time & death time of the trematodes. It was found that PEJG effectively paralyzed and killed the trematodes at all concentrations in dose dependent fashion (p<0.001). Thus the efficacy of the extract against trematodes (helminthes) was determined as well as traditional claim of the plant was also established.

Keywords: Death time, *Jatropha gossypifolia,* paralysis time, *Paramphistomum explanatum*, traditional use, trematode

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INTRODUCTION

In modern world, importance of helminths and infections due to them is reflected in such facts that one-third of people living in developing regions of Sub Saharan Africa, Asia and America are known to be infected with more than single helminth. Severity of such infection is further concerning in the facts that school going children, adolescents and pre-school children are more prone to helminth infection resulting in growth retardation, diminution in physical fitness, impairment in memory and cognition etc. In adults, onchocerciasis appeared to be a primary cause for blindness and skin diseases, while lymphatic filariasis (LF) causes limb and genital deformities. Furthermore, LF, onchocerciasis, along with hookworm infection and schistosomiasis reduced worker productivity badly worldwide. In addition to global morbidity, helminth infections are observed to have direct or indirect effect on the development of malaria, HIV/AIDS in the developing countries [1].

Gastrointestinal helminthes are prevalent in ruminants (from 25%-92%). In Asia, particularly, paramphistome infections are widespread. The major organisms are trematodes like *Paramphistomum explanatum, Paramphistomum ichikawai, Paramphistomum microbothrium, Paramphistomum cervi* etc. Adult and immature paramphistoms are rarely treated with antihelminthic agents. This is due to the reason that the removal of infection is expected merely beneficial to the animals, though it may provide a prophylactic treatment for the intermediate host [2-4].

Combating helminth infection with drugs, though possible, currently available such agents are very limited and a few worth mentioning such compounds to treat helminth manifestation being albendazole, oxamniquine, praziquantel and ivermectin. Apart from these drugs, diethyl carbamazine (developed in the first half of twentieth century) and mebendazole (first marketed in 1972) are represented in different pharmacopoeias for combating the most common helminth infections worldwide. The limitation in the choice of drug to treat helminthiasis, inspires research to discover new drug entities with improved activities against certain selected helminths [1].

Jatropha gossypifolia Linn., belonging to family Euphorbiaceae, is well known as Belly-ache bush or Lal-bherenda. It is a gregarious shrub, with palmately 3-5 lobed leaves, and dark-red, crimson or purplish flowers. Leaf margins, petioles and stipules are covered with glandular hairs. Capsules are found to be about 9 mm long, 3-lobed, truncate at both ends, seeds are greyish red. Leaves are 20 cm long which are shining brown at the beginning and which turn green on maturity. The plant is cultivated in gardens majorly for ornamental purposes [5].

In the Indian traditional medicine, *Jatropha gossypifolia* is used frequently. Biological actions involve purgative, emetic, analgesic, antidiarrhoeal, treating veneral diseases, skin sores preventive and anti-cancerous. It is also beneficial for anemia, dyscrasia, vertigo and dysphonia. Decoction of bark is used as emmenagogue. Latex is applied to ulcers. Leaves are useful in carbuncles, eczema and itches and also used as a blood purifier. Seed oil is used as purgative, emetic and stimulant. It is also applied for ulcers, in the treatment of leprosy and is beneficial in worm infection [5].

The root of the plant is recommended for leprosy and used as an antidote for snake bite. The diterpenoids which are the major constituents of the roots were found to be the active principles of the plant. Jatrophone, a novel macrocyclic diterpenoid, was reported as a major cytotoxic, and tumor inhibitory constituents of the root. Three new antitumor derivatives of jatrophone, 2-α-hydroxyjatrophone, 2-β-hydroxyjatrophone, and 2-β-hydroxy-5,6-isojatrophone were also reported from roots. Three other diterpenoids - jatropholone A, jatropholone B and jatrophatrione were subsequently isolated [5]. A new coumarino-lignoid, called Venkatasin was also isolated from the whole plant [6].

The objective of the present work was to investigate the *in vitro* trematocidal (anthelmintic) activity of a petroleum ether extract of *Jatropha gossypifolia* root (PEJG) against the trematode, *Paramphistomum cervi* for the first time as well as to establish its traditional use.

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MATERIALS AND METHODS

Plant material

Roots of *Jatropha gossypifolia* plant were collected from a local plant supplier of Kolkata. The herbarium specimen was characterized and identified by Dr. K. Kartigheyan, taxonomist and Scientist, Central National Herbarium (CNH), Botanical Survey of India, Howrah, India (voucher specimen no: CNH/55/2011/Tech II/511). The voucher specimen was deposited in the herbarium of the Division of Pharmaceutical Chemistry, Department of Pharmaceutical Technology, Jadavpur University, for future reference.

The roots were initially washed thoroughly to remove the earthy materials from the body. The roots were then cut as per need to remove the traces of aerial part present so that no aerial part is present in the root sample. Then they were allowed to dry under shade. Then the roots were coarsely powdered for extraction process.

Preparation of extract

The powdered root was extracted using petroleum ether (60-80°C) (S D Fine Chemicals, Mumbai) as solvent in a Soxhlet apparatus. The solvent was removed under vacuum using rotary vacuum evaporator, and the crude extract (PEJG, 8% w/w) was stored in a desiccator.

Test organism

Live parasites (*Paramphistomum explanatum*) were collected from water buffalo after several washing in sterile 0.85% sodium chloride solution from freshly slaughtered hosts at a slaughter house in Pillkhana, Kolkata [7-8]. They were kept in 0.9% phosphate buffered solution (PBS)) as per method described by Hossain et al. [9]. The specimen were identified by Dr Shuvajit Chakraborty, Parasitologist, Platyhelminthes Section, Zoological Survey of India, Kolkata (Ref No: Platy/Enquiry/2013-14/4/10938).

Trematocidal activity

The trematocidal activity of PEJG was done according to Hossain et al. [9] with slight modifications. Briefly, the collected adult trematodes were incubated at 37±1°C in PBS media containing either no extract (control) or the test extract (PEJG), at four concentration levels (0.5, 1, 2, and 5 mg/ml), or the standard drug, albendazole, at a dose of 10 mg/ml (Standard, GSK, Mumbai) in PBS supplemented with 1% dimethylsulfoxide (DMSO). Six replicates of the experiments were performed for each concentration. The time required for complete inactiveness or paralysis (paralysis time) and finally death of the helminthes were recorded. After being removed from the experimental medium, trematodes were dipped in PBS at 37±1°C and observed for movement on gentle stimulation; the paralyzed parasite either started movement or showed immobility. Death of the helminth was confirmed when it completely lost its motility even when vigorously shaken or dipped in warm water (50°C) followed by fading away of their body colour. Six replicates were used for each concentration.

Phytochemical analysis of the plant extract

Qualitative phytochemical analysis of PEJG was carried out by conventional methodologies in search of active ingredient responsible for trematocidal activity. The phytochemicals were included under the study for different secondary metabolites like alkaloids, anthraquinone glycosides, cardiac glycosides, lipids, tannins, steroids, flavonoids and saponins as per methodologies described by Harborne [10] and Khandelwal [11].

Statistical analysis

All experimental results were expressed in the form of mean ± standard error mean (S.E.M) and also analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism (Version 4.03, Graph Pad Software Inc) statistical software. In all cases, *p* value less than 0.05 was considered as threshold of statistically significant.

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RESULTS

Trematocidal activity

Trematodes incubated in control medium having PBS showed spontaneous substantial behavior for more than 24 hours. All trematodes incubated in the media containing PEJG, were found paralyzed within short period and finally killed in all concentration of PEJG with high significant value (p<0.001) (Table 1). Trematodes incubated in PEJG showed a decline in the motility resulting in their death. As per Table 1, enhancement of the the paralytic time as well death time was observed with reduction of the concentration in a dose dependent fashion. The best result was observed for PEJG at 25 mg/ml where it killed the trematodes within 158.83 ± 4.94 min. Enhancement of spontaneous movement of the trematodes was observed with standard (Albendazole treated group) at the beginning minutes.

Table 1: Trematocidal (anthelmintic) activity of petroleum ether extract from roots of *Jatropha gossypifolia* (PEJG) on *Paramphistomum explantum*

Treatment	Concentration (mg/ml)	Time (min) taken for paralysis and death of the trematodes#	
		Paralysis	Death
Control	0.0	Nil	Nil
PEJG	1.0	261.50 ± 5.90***	276.67 ± 5.68***
	2.0	205.67 ± 5.20***	223.33 ± 5.99***
	5.0	182.67 ± 3.67***	204.00 ± 3.44***
	10.0	153.17 ± 6.79***	176.67 ± 3.80***
	25.0	80.17 ± 3.72***	158.83 ± 4.94***
Albendazole	10.0	240.33 ± 17.09***	268.17 ± 15.92***

Result were expressed as the mean of results in 6 trematodes ± S.E.M. ***p < 0.001. Trematodes incubated in control medium showed spontaneous physical activities for more than 24 hours.

Phytochemical analysis

On qualitative phytochemical analysis of PEJG it was found that it possesses presence of cardiac glycosides, tannins, steroids as well as lipids.

DISCUSSION

In this study, *in vitro* trematocidal activity of PEJG was evaluated. The effectiveness of the extract is judged by loss of spontaneity of movement, considered as paralytic time followed by death of the trematodes [8]. Dose dependent efficacy was observed in all concentrations of PEJG in case of paralytic time as well death time. It was also found that the time period difference between paralysis and death was reduced with the lowering of concentration (p<0.001) due to the toxic effect of the extract which was most important for finding efficient trematocidal (anthelmintic) medicine.

In previous studies it was established that different types of secondary metabolites found in various plant derived extracts were responsible for anthelmintic affect as well trematocidal activity in different species [8, 12-15]. From the phytochemical evaluation it can be proposed that the secondary metabolites like steroids and cardiac glycosides were found in PEJG may be responsible for its potent trematocidal activity.

CONCLUSIONS

According to the results obtained from the present study it was confirmed that the petroleum ether extract of *Jatropha gossypifolia* root (PEJG) possess significant trematocidal activity (anthelmintic activity) against *Paramphistomum explanatum in vitro*. Thus, the traditional use of the plant is also established. The plant extract may have very potential new chemical entities which may be helpful for further development of novel trematocidal (anthelmintic) drugs in coming future.

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