IN VITRO ANTHELMINTIC ACTIVITY OF SEED EXTRACT OF *ARTOCARPUS LAKOOCHA* ROXB

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Summary

The ethanol extract of seeds of *Artocarpus lakoocha* Roxb. (ESAL) was investigated for anthelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (10-50 mg/ml) of seed extract was tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas DMF (Di-methyl formamide) as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintic activity at highest concentration of 50 mg/ml. The result shows that seed extract possesses vermicidal activity and found to be effective as an anthelmintic. The anthelmintic activity of ethanol extract of the seeds of *Artocarpus lakoocha* has therefore been demonstrated for the first time.

Key words: Anthelmintic Activity, *Artocarpus lakoocha*, *Pheretima Posthuma. Ascardia Galli*, *Raillietina spiralis*,

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Introduction

Helminthiasis, or worm infestation, is one of the most prevalent disease and one of the most serious public health problems in the world. Hundreds of millions if not billions of human infections by helminthes exist worldwide and with increased world travel and immigration from the developing countries (1).

Artocarpus lakoocha Roxb. (Syn: *A. lacucha* Buch.-Ham.) is a member of the family Moraceae. It is large deciduous tree reaching 15-18 m in height with a spreading head. It is a valuable tropical tree species native to India, Burma, Malay, Sri Lanka and West Peninnsula, and is also cultivated in Uttar Pradesh, Bengal, Khasi Hills and Western Ghats of India for fruit, furniture, timber, and feed. The plant is known as Monkey Jack in English and in Ayurveda called as Lakuch, Kshudra Panas, Granthiphala and Pitanaasha (2).

Reviews of the records in both, traditional and scientific literature indicated that *Artocarpus lakoocha* Roxb. have many medicinal uses. The infusions of bark when applied externally, draws out the purulent matter; heals boils, cracked skin and pimples. The unripe fruit is hot, sweet, sour; causes constipation, impotency, loss of appetite, eye troubles and blood complaints, whereas the ripe fruit causes aphrodisiac, improves taste and appetite. The lakoocha fruits are generally eaten fresh. The raw fruits and male flowers spikes (acidic and astringent) are utilized in pickles and chutney. The edible fruit pulp is believed to acts as a tonic for the liver. Lakoocha seeds and milky latex is good purgative and vermifuge (2).

The previous scientific reports suggest that, two new stilbene derivatives, lakoochins A and B, were isolated from the roots of *A. lakoocha*. Both exhibited antimycobacterial activity and showed cytotoxic activity against some cell lines (3). The brown powder called Puag-Haad in Thailand is a product of the aqueous extraction of *A. lakoocha* prepared by boiling the wood chips and then evaporating water away. This preparation has been used as a traditional anthelmintic drug for treatment of tapeworm infection in Thailand (4, 5). The stem bark contains oxyresveratrol, used for tapeworm. A lectin, artocarpin, isolated from seeds, precipitates several galactomannans. It agglutinates rat lymphocytes and mouse ascites cells (6). However, literature survey revealed that the seed extract has yet not been screened for its traditional claim of anthelmintic activity and hence an attempt has been made in the present study to investigate an anthelmintic activity of ethanol extract of seeds of *Artocarpus lakoocha*.

Materials and Methods

Plant materials and preparation of the extract

Fresh seeds of *Artocarpus lakoocha* were collected from Sirsi, Uttara Kannada District, Karnataka, India during May 2009. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen no. 110a is deposited in the herbarium of our institute. Fresh seeds were collected and dried. The coarse powder of the seeds (200 g) was soaked in 95% ethyl alcohol and extracted for 72 h at 60-65 ^{0}C. The solvent from the total extract was filtered; the concentrate was evaporated to dryness under

reduced pressure and low temperature on a rotary evaporator to give the ethanolic extract (10 % w/w yield). The extract was formulated as 10, 20 and 50 mg/mL solutions in DMF (di–methyl formamide) and investigated for anthelmintic activity.

Drugs and chemicals

Piperazine citrate (Glaxo Smithkline) was used during the experimental protocol.

Phytochemical screening

Freshly prepared ethanolic extract of the seeds of *A. lakoocha* (ESAL) was subjected to preliminary phytochemical screening for detection of major chemical constituents (7).

Investigation of anthelmintic activity

The ethanol extract of the seeds of *A. lakoocha* (ESAL) was investigated for its anthelmintic activity against earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (25, 50 and 100 mg/mL) of the extract was tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. The anthelmintic activity was carried as per the method of Ajaiyeoba et al (8) with minor modifications. The anthelmintic activity was evaluated on the adult Indian earth warm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (9-11). Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. The earthworms of 5-7 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Use of *Raillietina spiralis* and *Ascaridia galli* as a suitable model for screening of anthelmintic drug was advocated earlier (12, 13).

Table 1. *In-vitro* anthelmintic activity of ethanol extract of the seeds of *Artocarpus lakooocha* (ESAL)

Group	Treatm-ent	Conce-ntration (mg/ml)	*Pheretima posthuma* (Earthworm)		*Raillietina spiralis* (Tapeworm)		*Ascardia Galli* (Rounrworm)	
			P	P	P	D	D	D
I	Control (DMF)	-	-	-	-	-	-	-
II	ESAL	10	29±0.48	27±0.85	25±0.51	65±0.21	61±2.13	70±1.28
III	ESAL	20	20±0.61	18±0.56	17±0.26	41±1.23	43± 1.64	48±1.61
IV	ESAL	50	09±0.32	10±0.20	08±0.15	24±0.94	25±0.98	29±1.05
V	Standard (PZC)	10	07±0.29	08±0.11	06±0.09	14±0.24	09±0.25	20±0.69

Each value represents mean ± SEM (n=6). DMF: Di-methyl-formamide, PZC: Piperazine citrate, P: Time taken for paralysis of worms (min), D: Time taken for death of worms (min).

Test samples of the extract was prepared at the concentrations, 10, 20 and 50 mg/ml in DMF and six worms i.e. *Pheretima posthuma, Raillietina spiralis* and *Ascaridia galli* of approximately equal size (same type) were placed in each 9 cm petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg/ml) was used as reference standard and DMF as control. This procedure was adopted for all three different types of worms (14-17). All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 ^{0}C). All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

Results and Discussion

From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. The ethanol extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration, for all three types of worms. However, extract exhibited prominent activity at lower concentration (10 mg/ml) against all three types of worms. Evaluation of anthelmintic activity was compared with reference standard piperazine citrate, which was more potent than the seed extract activity (Table.1).

Preliminary phytochemical screening of extract revealed the presence of flavonoids and other phenolic compounds. Phenolic compounds show anthelmintic activity (18). It is possible that phenolic contents in the extract of seeds of *A. lakoocha* produced similar effects.

From the above results, it is concluded that *Artocarpus lakoocha* seeds used by tribals traditionally to treat intestinal worm infections, showed promising anthelmintic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as anthelmintic. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

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