

**IN VITRO ANTHELMINTIC ACTIVITY OF ROOT OF
HOMONOIA RIPARIA Lour. AGAINST *PHERETIMA POSTHUMA***

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Summary

In present study Aqueous extract of *Homonoia riparia* (HRAE) and Ethanol extract of *Homonoia riparia* (HREE), at different concentrations (25-200 mg/ml) were evaluated for their invitro anthelmintic activity against adult Indian earth worms, *Pheretima posthuma* (Pheritimidae). The anthelmintic assay involves determination of paralysis time and death time of worms. Albendazole (20 mg/ml) was used as a standard reference and normal saline as a normal control and 10% propylene glycol in normal saline as a experimental control. Results showed that both HRAE and HREE are having significant anthelmintic activity, but HREE is more potent as compared to HRAE as it took less time to cause paralysis and death of earthworms.

Keywords: *Homonoia riparia* Lour., Paralysis time, Death time.

Introduction

Helminthiasis is a worm infestation and highly prevalent disease particularly in third world countries due to poverty, illiteracy, lack of adequate sanitary facilities and pure water supply(1). The helminthes are multicellular organisms possessing three germ layers and exhibiting a bilateral symmetry. They are classified into round worms, tape worms and flukes. Worms cause various GI and general symptoms. In addition, some of them can cause blood loss, nutritional deficiencies, urticaria and blood loss and other allergic manifestations and even intestinal obstruction (2). The plants are known to provide a rich source of botanical anthelmintics (3). A number of plants have been reported to treat helminthiasis like *Enicostemma littorale* (4), *Cocos nucifera*(5), *valeriana wallichii* DC (6), *Eupatorium odoratum* (7).

Homonoia riparia Lour. (Euphorbiaceae) is a shrub attaining height of 1 to 3 meters. The leaves are linear-lanceolate, 12 to 20cm long and 1.5 to 2 cm wide; upper surface is green and shining, and lower surface brown and hairy (8). This is one of the controversial drugs in Ayurveda (9). It is widely distributed in India, China, Malaysia, Indonesia and Philippines. It is usually inhabiting in rocky river beds (10-13).

In traditional medicine, the root is good for ulcer, strangury, urinary discharges, and vesical calculi. A decoction is given in piles, stone in bladder, gonorrhoea and used as diuretic (14). *Homonoia riparia* was reported to possess antiurolithiatic activity (15) and diuretic activity (16), twelve compounds were isolated from the ethyl acetate extract of root of *H.riparia* that includes tannins also (17). Tannins in several plants reported to show anthelmintic property (18,19). Since the tannins like gallic acid are present in the root of *H.riparia* the present study was taken up to prove the anthelmintic activity and literature reveals that no such work is carried on it.

Material and Method

Collection

The plant material proposed to study was collected from the Thenamalai forest, Kerala and was authenticated by S.N Yoganarashiman, Research co-ordinator in M.S Ramaiah College of pharmacy and prepared herbarium specimen, provided specimen number 27 and preserved in the Museum of M.S Ramaiah College of Pharmacy. The roots were sun dried and stored at room temperature in a gunny bag.

Preparation of Root Extract

Ethanol extract: The powdered plant material was extracted with Ethanol using Soxhlet apparatus, a hot Soxhlet method. Then solvent was cooled at room temperature, filtered and evaporated to dryness.

Aqueous Extract: The powdered plant material was extracted with Chloroform water (1:1000) by cold maceration method, filtered and evaporated to dryness.

Reference drug: Albendazole was prepared by dissolving them in normal saline at concentration of 20 mg/ml.

Experimental control treatment: A 10% propylene glycol in normal saline was used as experimental control treatment.

Normal control: Saline was prepared and used to treat the normal control group.

Anthelmintic Activity

The Earth worms *Pheretima posthuma* nearly equal size (3.5-5cm) collected from University of Agricultural sciences, Bangalore was used for evaluating the anthelmintic activity of root extracts using reference

substance for comparison. These were procured from local suppliers GSK Pharmaceuticals Ltd, Tumkur road, Nelamangala and maintained at Gautham college of pharmacy, Bangalore.

Activity against Earth Worms

Anthelmintic activity was performed according to the method (20). One adult Indian earth worm *Pheretima pothuma* as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Pheretima pothuma* was placed in petridish containing four different concentrations (25, 50, 100, 200 mg/ml) each of HRAE and HREE. Each petridish was placed with 6 worms and observed for paralysis (or) death. Observations were made up to 4 hrs of test period for the time taken to paralysis or death of individual worms. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm(min) was recorded after ascertaining that worm neither moved when shaken nor when given external stimuli with fading away their color. In the same manner Albendazole was included as a reference compound. The test results were compared with reference standard Albendazole (20 mg/ml) treated sample.

Statistical Analysis

All values represent Mean \pm SEM; n=6 in each group. Control group worms were alive up to 24 hours of the experiment. Statistically analyzed by using one way of ANOVA.

Results

As per Table I, the crude extract samples which were used to evaluate anthelmintic activity, showed variable death time and paralysis time at different concentrations and the mean time values were calculated for each parameter. The both HRAE and HREE showed significant death at higher concentrations. The HRAE showed significant activity at two doses i.e at 100 mg/kg and 200 mg/ml where all 6 worms were died, whereas HREE also showed death of all 6 worms at both doses of 100 mg/kg and 200 mg/ml. But time taken to show anthelmintic activity at 100 mg/kg is more when compared to 200mg/kg. In HRAE at 50mg/kg only 2 worms were mortal and in HREE only 1 worm was mortal. In both HRAE and HREE at 25 mg/ml did not show any mortality and paralysis. The paralysis time of HRAE at 100 and 200mg/ml were significant, and HREE also showed significant paralysis time at 200 mg/kg only.

Discussion

The helminthiasis activity of the worm according to the result ethanol > aqueous extracts whereas paralysis activity of worm also indicates ethanol > aqueous extract. The HREE has more anthelmintic activity than HRAE, but the HREE exhibit significant paralysis time than the HRAE. This is due to increased level of tannins in the ethanol extract. The degree of helminthiasis of different extract is due to the level of tannins present in the root of *Homonoia riparia* Lour. Tannins in several plants reported to show anthelmintic property (18,19). Tannins, the polyphenol compounds are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or binds to the glycoprotein on the cuticle of parasite and cause death.

Table I. Invitro anthelmintic activity of Aqueous and Ethanol extract of root of *Homonoia riparia* Lour.

Extract of <i>H.riparia</i>	Conc. mg/ml	Paralysis time (min.)	Death time(min.)
Normal Control	0	0	0
Experimental control	0	0	0
Albendazole	20	38.3±9.08	56±6.66
HRAE	25	0.0±0.0	0.00±0.00
	50	145±23.00	165±9.61
	100	51.66±69.3	65.00±87.25
	200	46.66±10.36	55.0±13.50
HAEE	25	0.0±0.0	0.0±0.0
	50	195±4.123	220±58.26
	100	158±6.123	148.33±21.88
	200	41.66±4.18	51.66±8.66

All values represent Mean±SEM; n=6 in each group. Control group worms were alive up to 24 hours of the experiment. Statistically analyzed by using one way of ANOVA.

Conclusion

HRAE showed significant anthelmintic effect at the dose of 100 and 200 mg/ml whereas HREE showed significant anthelmintic activity at 200mg/ml. It concludes that both extracts show significant activity at high dose only. This anthelmintic activity may be due to presence of Tannins in the root of *Homonoia riparia* but the exact tannins showing this activity is unknown and further studies must be carried on to find out it.

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