



RESEARCH ARTICLE

***In-Vitro* Anthelmintic Activity of *Ehretia laevis* bark On Indian Adult Earthworm**

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Manuscript No: IJPRS/V7/I4/00012, Received On: 19/11/2018, Accepted On: 30/11/2018

ABSTRACT

The development of anthelmintic resistance and the high cost of conventional anthelmintic drugs it leads to the evaluation of medicinal plants as an alternative source of anthelmintics. In the current study, *in-vitro* experiments were conducted to determine the possible anthelmintic effects of crude Methanolic, aqueous and hydroalcoholic extracts of the bark of *Ehretia laevis* on eggs and adult *Pheritima posthuma* and *Ascaridia galli*. Three concentrations (10, 25, 50 mg/ml) of Methanolic, aqueous and hydroalcoholic extracts of plant *Ehretia laevis* were studied in activity which involved the determination of time of paralysis (vermifuge) and time of death (vermicidal) of the worms. Piperazine citrate in same concentration as those of extract was included as standard reference and normal saline water with 1% CMC as control. All the extracts exhibited significant anthelmintic activity at a concentration of 50 mg/ml. Peak activity was exhibited by the methanolic extract at a concentration of 50 mg/ml. The overall findings of the present study have shown *Ehretia laevis* contain possible anthelmintic compounds and further evaluation of these plants should be carried out. The ethnomedicinal claim of anthelmintic activity of this plant is genuine.

KEYWORDS

Anthelmintic activity, *Ehretia laevis*, *Pheritima posthuma*, *Ascaridia galli*, Piperazine citrate, Helminthiasis

INTRODUCTION

Parasitic infection including Helminthiasis is a critical serious problem in the tropical regions including the Asian and African countries which affects more than 2.5 billions of people worldwide. Helminths produce serious problem in human beings and other animals around the world specifically to the third world countries¹. Different type of helminths infects the human and animals out of which intestinal round worms *Pheritima posthuma* (Annelida) are most common. Approximately 200 million

people suffer severe morbidity associated with these parasites and half of which are school-going children affected by massive infections. Variety of several clinical symptoms arises due to this infection include dysentery, diarrhoea, nausea, vomiting, loss of appetite, loss of weight, acidity and anaemia. Other sign and symptoms of helminthic infections include respiratory symptoms, dermatological consequences and epilepsy as a result of neurocysticercosis. Helminthic infections may also subvert immune responses to pathogens of other diseases such as tuberculosis, HIV, and malaria². Although the majority of infections are due to the worms generally limited to tropical regions, they can also occur to travellers who have visited those areas and some of them can develop in temperate

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climates³. Helminthiasis is a disease in which a part of the body is infested with worms like as pinworm, roundworm or tapeworm. Typically, the worms present in the gastrointestinal tract but may also reside into the liver and other organs, infected peoples are excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation⁴. Other peoples can be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases can cause severe morbidity, including filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis⁵. As per the WHO survey only synthetic drugs are sometimes used in the treatment of helminth infestations in human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effect. In view of this, an attempt has been made to study the anthelmintic activity of herbal drug. Development of resistance to most of the commercially available anthelmintics drugs are became a severe problem worldwide. Sometimes, these drugs are unaffordable, inaccessible or inadequately available to the resource poor farmers of the developed and developing countries⁶. These factors paved the way for herbal remedies as alternative anthelmintics⁷. Therefore the evaluation of the activities of medicinal plants claimed for possessing the anthelmintic property is getting attention these days⁸. Screening and proper evaluation of the claimed medicinal plants as anthelmintics could offer possible alternatives that may be both sustainable and environmentally acceptable⁹. *Ehretia laevis* is fast-growing small tree belonging to family Ehretiaceae. The plant is native to India, Pakistan, Laos, Myanmar, Vietnam, China, Bhutan. The plant *Ehretia laevis* is located at hilly forests, in ravine and on hill slopes. The plant is known as Dant-Rang, Vadhvarni, Chamror¹⁰. The inner bark of *E. laevis* is used as food. Leaves are applied to ulcers, skin diseases and in headache. Fruit is used as urinary passage, lung and spleen diseases, astringent, anthelmintic, diuretic, demulcent, expectorant. Powdered kernel mixed with oil is

a remedy in ringworm. Seeds are anthelmintic. Barks are used in throat infection. Root for veneral diseases. The plant contains chemical constituents likes fatty acids, phenolic acids, flavonoids, cyanogenetic glycosides, and benzoquinones^{11,12}.

In the current study, we have attempted to investigate methanolic, hydroalcoholic and aqueous extracts of bark of medicinal plant *Ehretia laevis* for their claimed anthelmintic activity.

MATERIALS AND METHOD

Plant Collection

The fresh barks of plant *Ehretia laevis* were collected from haripura and manudevi region of Taluka Yawal, District Jalgaon, India. The selected plants were authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule, Maharashtra. Barks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Selection of Experimental Worms

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the experiment. *Pheretima posthuma* is commonly known as earthworm and were collected from water logged areas. *Ascaridia galli* is nematode were obtained from freshly slaughtered area. Both worms were identified by PG Department of Zoology, SSVPS's Science College, Dhule. Worms were washed with normal saline to remove all faecal matter. The earthworms of 7-9 cm in length and 0.2-0.4 cm in width were used for all the experimental protocol. Ready availability, anatomical and physiological resemblance of *Pheretima posthuma* and *Ascaridia galli* made it to be used initially for *in-vitro* evaluation of anthelmintic activity.

Preparation of Plant extract

The bark of plant were thoroughly washed with tap water, dried at room temperature and transformed to coarse powder. The bark powder were extracted with three solvents i.e methanol,

water and water-ethanol separately by Soxhlet extraction method. Finally, the extract was evaporated and dried under vacuum to obtain thick sticky extract.

Drugs and Chemicals

Piperazine citrate [Actepar Syrup], Methanol, Distilled water, Ethanol and were used during the experimental protocol. All the chemicals used are laboratory and analytical grade.

Experimental Work^{13,14,15,16}

The anthelmintic activity was carried out as described by *Ajaiyeoba EO. et. al*, 2001, with minor modifications. The assay was performed on adult Indian earthworm *Pheritima posthuma* and *Ascardia galli* due to their anatomical and physiological resemblance with the intestinal round worm parasite of human being^{17,18}. Because of easy availability, earth worms have been used widely for initial evaluation of anthelmintic compounds in vitro. The Indian earthworm *Pheritima posthuma* and *Ascardia galli*, of nearly equal size, six in each group was taken for the experiment. The methanolic, aqueous and hydroalcoholic dried extract were suspended in 1% w/v Carboxy Methyl Cellulose, prepared in normal saline water in three different conc. (10, 25 and 50 mg/ml). Piperazine citrate suspension of concentration 10mg/ml was taken as standard and normal saline water with 1% CMC was taken as a control. Worms were placed in petridish containing 25 ml of sample (drug) solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water (50°C). Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

Statistical Analysis¹⁹

The data presented as Mean \pm SEM. The activities of both the leaves extracts were compared with the control. All the extracts showed significantly higher duration of paralysis and death. Values of $P < 0.001$ were considered statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical screening of methanolic, aqueous and hydroalcoholic extract of bark of plant *Ehretia laevis* were revealed the presence of glycosides, alkaloids, saponins, flavonoids and tannins. As shown in Table 1, methanolic extract exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration, for *Pheritima posthuma* and *Ascardia galli* worms. The methanolic, aqueous and hydroalcoholic extracts showed paralysis followed by death of the worms at all tested dose levels. The potency of the extracts was found inversely proportional to the time taken for paralysis of death of worms.

The methanolic extract of bark of plant *Ehretia laevis* caused paralysis is 10.51 min and time of death is 20.27 min, hydroalcoholic extract revealed paralysis of 26.76 min and time of death 38.42 min while aqueous extract revealed paralysis of 35.37 min. and time of death 47.16 min. respectively against *Pheritima posthuma* at 50mg/ml concentration The reference drug Piperazine citrate showed the paralysis at 2.5 min. and time of death at 10 mg/ml conc. 16.23 min. respectively. The methanolic extract of bark of plant *Ehretia laevis* caused paralysis is 12.52 min and time of death is 23.31 min, hydroalcoholic extract revealed paralysis of 27.14 min and time of death 37.20 min while aqueous extract revealed paralysis of 32.71 min. and time of death 45.59 min. respectively against *Ascardia galli* at 50mg/ml concentration The reference drug Piperazine citrate showed the paralysis at 3.67 min. and time of death at 10 mg/ml conc. 18.29 min. respectively.

Piperazine citrate by increasing chloride ion conductance in worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis²⁰. The extracts of bark of plant *Ehretia laevis* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical screening of the extracts

revealed the presence of tannins among the other chemical constituent within them. The chemical constituent tannins were shown to produce anthelmintic activities²¹. Chemically tannins are polyphenolic compounds²². Some synthetic phenolic anthelmintics e.g. Albendazole, Niclosamide, Oxiclozanide, Bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation²³. It is possible that tannins contained in the extracts of bark of plant *Ehretia laevis* produced similar effects. Another possible anthelmintic effect of tannins is that they can be bind to free proteins in the gastrointestinal tracts of host animal²⁴ or

glycoprotein on the cuticle of the parasite²⁵ and may cause death.

The traditional medicinal plants hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in developed and developing countries, including in India, Pakista, Srilanka, Bhutan It is in this context that the people consumed several plants or plant based preparation to cure helminthic infections²⁶. The origin of many effective herbal drugs has been found in the traditional medicines practices and in view of this it is important to undertake studies pertaining to screening of the folklore medicinal plants for their claimed anthelmintic efficacy.

Table 1: Anthelmintic activity of methanolic, aqueous and hydroalcoholic extract of bark of plant *Ehretia laevis* on *Pheretima posthuma*

Extract	Concentration mg/ml	<i>Pheretima posthuma</i>	
		Time of Paralysis (P)	Time of Death (D)
Control (1% CMC)	---	---	---
Standard (Piperazine citrate)	10 mg/ml	2.50 ± 0.13	16.23 ± 0.58
Methanolic extract	10 mg/ml	23.71 ± 0.49	37.47 ± 0.37
	25 mg/ml	14.77 ± 0.34	27.26 ± 0.39
	50 mg/ml	10.51 ± 0.36	20.27 ± 0.29
Hydralcoholic extract	10 mg/ml	41.49 ± 0.39	61.94 ± 0.50
	25 mg/ml	35.12 ± 0.50	45.82 ± 0.33
	50 mg/ml	26.76 ± 0.44	38.42 ± 0.20
Aqueous extract	10 mg/ml	52.15 ± 0.26	72.34 ± 0.40
	25 mg/ml	42.90 ± 0.28	63.41 ± 0.24
	50 mg/ml	35.37 ± 0.41	47.16 ± 0.62

All Values represent Mean± SEM; n=6 in each group. Comparisons made between standard versus treated groups, P<0.05 was considered significant

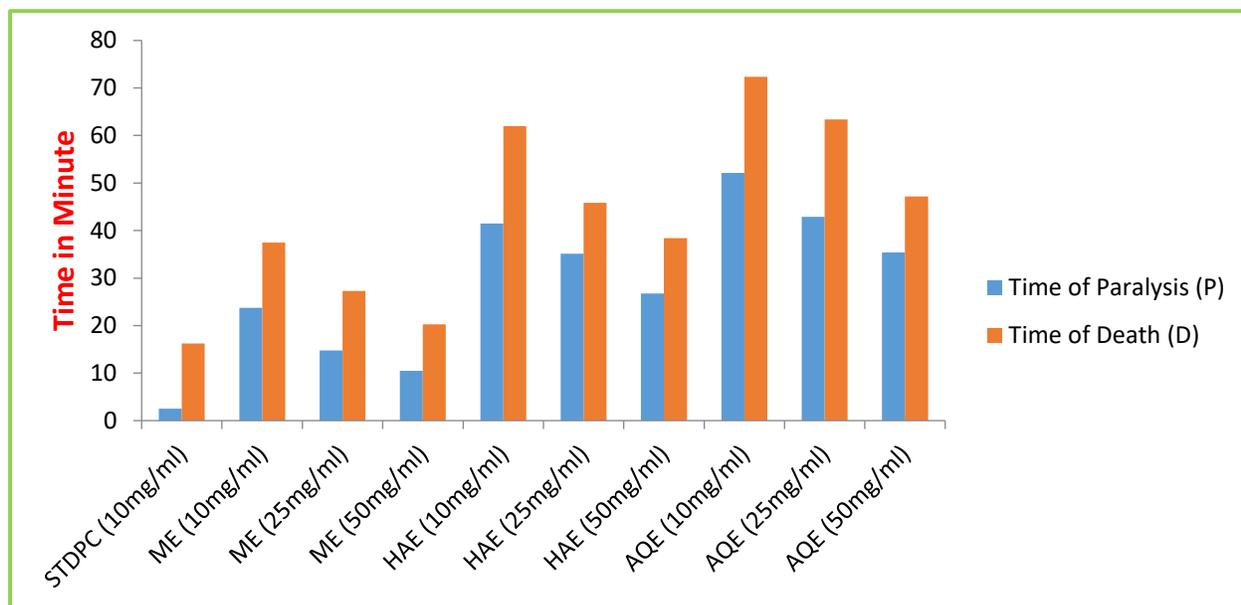


Figure 1: Anthelmintic activity of *Ehretia laevis* bark on *Pheretima posthuma*

Table 2: Anthelmintic activity of methanolic, aqueous and hydroalcoholic extract of bark of plant *Ehretia laevis* on *Ascaridia galli*

Extract	Concentration mg/ml	<i>Ascaridia galli</i>	
		Time of Paralysis (P)	Time of Death (D)
Control (1% CMC)	---	---	---
Standard (Piperazine citrate)	10 mg/ml	3.67 ± 0.35	18.29 ± 0.37
Methanolic extract	10 mg/ml	21.34 ± 0.30	32.40 ± 0.45
	25 mg/ml	18.42 ± 0.17	28.93 ± 0.22
	50 mg/ml	12.52 ± 0.31	23.31 ± 0.30
Hydralcoholic extract	10 mg/ml	43.57 ± 0.48	65.54 ± 0.60
	25 mg/ml	32.31 ± 0.36	43.60 ± 0.48
	50 mg/ml	27.14 ± 0.62	37.20 ± 0.56
Aqueous extract	10 mg/ml	48.15 ± 0.46	69.62 ± 0.30
	25 mg/ml	40.54 ± 0.43	59.00 ± 0.22
	50 mg/ml	32.71 ± 0.41	45.59 ± 0.30

All Values represent Mean± SEM; n=6 in each group. Comparisons made between standard versus treated groups, P<0.05 was considered significant

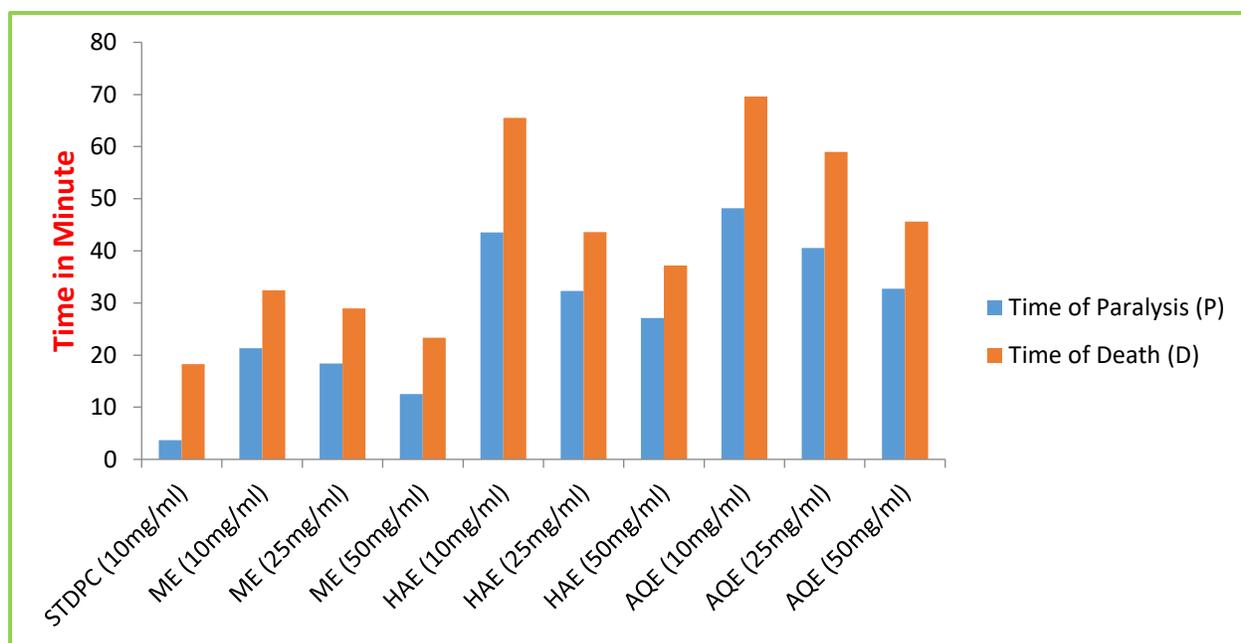


Figure 2: Anthelmintic activity of *Ehretia laevis* bark on *Ascardia galli*

CONCLUSION

The results of the present study clearly indicated that the methanolic extract of plant *Ehretia laevis* produce anthelmintic activity against Indian earthworm *Pheretima posthuma* and *Ascardia galli*. The plant possesses significant anthelmintic activity at 50 mg/ml concentration measured by time taken for paralyse and death of the earth worms. The current investigation leads to conclusion that the bark of plant *Ehretia laevis* have potent anthelmintic activity when compared with the conventionally used drug. Further studies can be explore by using *in vivo* models and to carry isolation of active constituents from methanolic extract and establishment of the effectiveness and pharmacological rational for the use of plant *Ehretia laevis* as an anthelmintic drug.

ACKNOWLEDGEMENT

The Corresponding author is very grateful to Principal and my PhD guide, Dr. Rajendra D. Wagh, DCS's A.R.A. College of Pharmacy, Nagaon, Dhule, for providing necessary facilities, support and proper guidance.

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HOW TO CITE THIS ARTICLE

Joshi, U. P., Wagh, R. D. (2018). In-Vitro Anthelmintic Activity of *Ehretia laevis* bark On Indian Adult Earthworm. *International Journal for Pharmaceutical Research Scholars*, 7(4), 22-30. <http://dx.doi.org/10.31638/IJPRS.V7.I4.00012>

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