IJMPR 2023, 7(7), 38-43

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

IN VITRO ANTHELMINTIC ACTIVITY OF *ALBIZIA LEBBECK* (L.) BENTH. BARK ON INDIAN ADULT EARTHWORM

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Received on: 27/04/2023
Revised on: 17/05/2023
Accepted on: 07/06/2023

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ABSTRACT

Objective: Development of anthelmintic resistance and high cost of conventional anthelmintic drugs leads to the evaluation of medicinal plants as another source of anthelmintics. Gastrointestinal nematodes are the major restrictive factor for the successfulness of livestock production all over the world. Appearance of resistance strains as well as scarcity and high cost of the currently available drugs has lead to the evaluation of other alternative helminth control options, mainly from plants. The development of anthelmintic activity and the high cost of synthetic 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 Chloroform, Methanolic, Aqueous and Hydroalcoholic extracts of the bark of the plant *Albizia lebbeck* (L.) Benth. on eggs and adult *Pheretima posthuma* and *Ascaridia galli.* **Methods:** Three concentrations (10, 25 and 50 mg/ml) of chloroform, methanolic, aqueous and hydroalcoholic extracts of bark of plant *Albizia lebbeck* (L.) Benth. were studied in activity which involved the determination of the time of paralysis (called as vermifuge) and time of death (called as vermicidal) of the worms. Piperazine citrate in same concentration as those of extract was included as standard reference and normal saline water with 1% carboxymethyl cellulose as control. **Results:** The chloroform, methanolic, aqueous and hydroalcoholic 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. **Conclusion:** The overall findings of the present study have shown that *Albizia lebbeck* (L.) Benth. contain possible anthelmintic compounds and further evaluation of these plants should be carried out. The traditional use of anthelmintic activity of this plant is genuine.

KEYWORDS: *Albizia lebbeck* (L.) Benth., *Pheretima posthuma*, *Ascaridia galli*, Piperazine citrate, *Helminthiasis,* Anthelmintic etc.

INTRODUCTION

Helminths are the most familiar infectious agent for human being in developing countries and produce a worldwide burden of disease and leads to the prevalence of eosinophilia, malnutrition, anaemia and pneumonia. The disease is greatly prevalent particularly in third world countries.[1]

There are different types of helminths that infects humans and animals in which intestinal roundworms *Pheretima posthuma* (Annelida) are most common. Variety of several clinical symptoms occurs due to this infection like as diarrhea, nausea, vomiting, dysentery, loss of appetite, loss of weight, acidity and anemia. Other signs and symptoms of helminthic infections are respiratory symptoms, dermatological consequences and epilepsy as a result of neurocysticercosis. Helminthic infections may also warn immune responses to pathogens of other diseases such as malaria, tuberculosis and HIV.[2]

Helminthiasis is a disease in which part of human body is infected with worms like as flatworms, roundworm or tapeworm. Typically, those worms are present in the gastrointestinal tract but may also be located into the liver and other organs, infected peoples are excreted helminth eggs in their feces, which then contaminate the soil in areas with inadequate sanitation Among these, helminth infections play a critical role in small ruminant production leading to economic losses, mainly loss of production through mortality, weight loss, reduced milk and meat production.[3]

Further peoples can be infected by ingesting eggs or larvae from contaminated food or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases can cause severe morbidity including schistosomiasis, filariasis (a cause of elephantiasis) and onchocerciasis (river blindness).[4]

As per the WHO, there are some synthetic drugs used in the treatment of helminth infection in human beings, but these synthetic drugs are away from millions of people and may have many side effects. Due to this, an effort has been taken to study the anthelmintic activity of medicinal plants. Development of resistance to most of the commercially available anthelmintics drug is became a severe problem worldwide.[5]

Besides anthelmintic resistance other risk of residue, unavailability and high cost especially to farmers of low income in developing countries diverted the researcher's attention toward the development of alternate methods for the treatment of Helminthiasis.[6] Screening and proper evaluation of medicinal plants for anthelmintic activity could offer possible alternatives that may be both sustainable and environmentally acceptable. The plants are known to provide a rich source of botanical anthelmintics.[7], [8]

Albizia lebbeck (L) Benth . (Family – Fabaceae) is commonly known as Lebbeck Tree, siris tree in English; shiris Tree, siris in Hindi. A large, erect, unarmed, deciduous, spreading tree. A. lebbeck (L.) Benth. Deciduous tree, growing to 30 m tall in native forests. In open situations, trees develop a spreading, sometimes multistemmed habit, to 25 m tall and 30 m across, with low branching. It can develop root suckers, and produces dense coppicing from cut stumps.[9]

Bark used as astringent, acrid, bitter, sweet, expectorant, aphrodisiac, depurative, ophthalmic, tonic[10], diseases of the gum and toothache[11], weakness, cures diseases of blood, anthelmintic, itching, skin disease, piles, deafness, scabies, syphilis and boils[12] helminthes infections, bronchitis, dental infections, leprosy, antidiarrheal activity and paralysis[13] pruritus, eczema, paralysis and worm infestation.[14] Flower used as Asthma[15] bronchitis and chronic cough[16] chronic catarrh, inflammation, poisoning, skin diseases, opthalmopathy, leprosy.[17] Leaves used as Antiseptic, anti-cancer activity, anti- tubercular, antimicrobial, anti- protozoal, anti- dysenteric and antifertility.[18] The seed oil is applied topically to cure leucoderma, astringent, aphrodisiac[19], diarrhoea and piles.[20]

In the current study, we have attempted to investigate chloroform, methanolic, hydroalcoholic and aqueous extracts of the bark of medicinal plant *Albizia lebbeck* (L.) Benth. for their claimed anthelmintic activity.

METHODS

Plant collection

The bark of medicinal plant *Albizia lebbeck* (L.) Benth. were collected from Laling region of District Dhule, India. The plant *Albizia lebbeck* (L.) Benth. were authenticated by Dr. D. A. Dhale, Asst. Professor, PG, and Research Department of Botany SSVPS's, L. K. Dr. P. R. Ghogrey Science College, Dhule, Maharashtra. Stembarks 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 such as *Pheretima posthuma* and *Ascaridia galli* were used to carry out the anthelmintic activity. *Pheretima posthuma* is commonly known as earthworm and were collected from waterlogged areas. *Ascaridia galli* is nematode and were obtained from freshly slaughtered area. Both worms were identified by the PG Department of Zoology, SSVPS's Science College, Dhule. Worms were washed with normal saline to remove all fecal matters. The earthworms of 6–8 cm in length and 0.3–0.5 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 medicinal plant *Albizia lebbeck* (L.) Benth. was thoroughly washed with tap water, dried at room temperature, and transformed to coarse powder. The powder of leaves was extracted with four solvents, i.e., chloroform, methanol, water and water-ethanol separately by Soxhlet extraction and maceration method. Finally, all the extracts were evaporated and dried under vacuum or tray dryer to obtain thick sticky extract.

Drugs and chemicals

Piperazine citrate (Actipar Syrup), methanol, distilled water, and ethanol were used during the experimental protocol. All the chemicals used are belonging to laboratory and analytical grade.

Experimental work[21-24]

The anthelmintic activity was carried out on earthworms which was well explained by Ajaiyeoba *et al.*, 2001, with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma* and *Ascaridia galli* due to their anatomical and physiological resemblance with the intestinal roundworm parasite of human being.[25, 26] Due to easy availability, earthworms have been used widely for initial evaluation of anthelmintic compounds *in vitro*. The Indian earthworm *Pheretima posthuma* and *Ascaridia galli*, of nearly equal size, six in each group, was taken for the experiment. The chloroform, methanolic, aqueous, and hydroalcoholic dried extract of bark of medicinal plant *Albizia lebbeck* (L.) Benth. Were suspended in 1% w/v carboxymethyl cellulose, prepared in normal saline water in three different conc. (10, 25, and 50 mg/ml). Piperazine citrate suspension of the concentration of 10 mg/ml was taken as standard, and normal saline water with 1% carboxymethyl cellulose was taken as a control. Worms were placed in Petri dish containing 25 ml of sample (drug) solution. Time for paralysis was noted and observations are made by any movement could not be seen except when the worms were shaken vigorously or

when dipped in warm water (60°C). Death was included when the worms lost their motility followed by white secretions and fading away of their body color.

Statistical analysis[27]

The data presented as mean ± standard error of the mean. The activities of bark extracts were compared with the control. All the plant extracts showed significant duration of paralysis and death. Values of $p<0.001$ were considered statistically significant.

RESULTS AND DISCUSSION

Table 1: Anthelmintic activity of Chloroform, Methanolic, Hydroalcoholic and Aqueous extract of the bark of medicinal plant *Albizia lebbeck* (L.) Benth. on *Pheretima posthuma*.

Extract	Concentration (mg/ml)	*Pheretima posthuma*	
		Time of paralysis (P)	Time of death (D)
Control 1% CMC Carboxymethyl cellulose	---	---	---
Standard (Piperazine citrate)	10 mg/ml	2.7±0.15	16.11±0.59
Chloroform extract	10 mg/ml	47.13±0.59	72.12±0.33
	25 mg/ml	31.62±0.34	61.48±0.52
	50 mg/ml	35.13±0.57	45.97±0.24
Methanolic extract	10 mg/ml	28.62±0.18	42.54±0.64
	25 mg/ml	16.49±0.45	35.47±0.56
	50 mg/ml	9.26±0.29	25.44±0.23
Hydroalcoholic extract	10 mg/ml	36.15±0.47	52.56±0.54
	25 mg/ml	27.18±0.28	42.49±0.63
	50 mg/ml	15.68±0.19	28.13±0.37
Aqueous extract	10 mg/ml	44212±0.58	68.12±0.34
	25 mg/ml	37.42±0.33	57.39±0.52
	50 mg/ml	30.04±0.57	42.78±0.21

All values represent Mean±SEM; n=6 in each group. Comparisons made between standard versus treated groups, $p<0.05$ was considered statistically significant. SEM: Standard error of the mean.

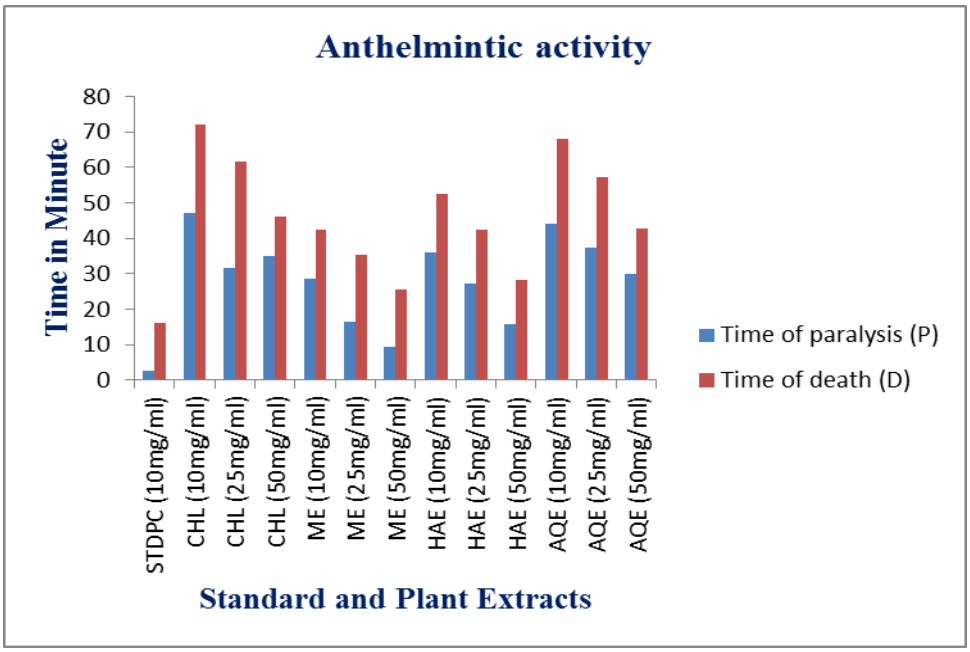

Fig. 1: Anthelmintic activity of *Albizia lebbeck* (L.) Benth. bark extracts on *Pheretima posthuma*.

Where, STDPC - Standard Piperazine citrate, CHL- Chloroform extract, ME - Methanolic extract, HAE - Hydroalcoholic extract, AQE - Aqueous extract

Table 2: Anthelmintic activity of Chloroform, Methanolic, Hydroalcoholic and Aqueous extract of the bark of medicinal plant *Albizia lebbeck* (L.) Benth. on *Ascaridia galli*.

Extract	Concentration mg/ml	*Ascaridia galli*	
		Time of paralysis (P)	Time of death (D)
Control 1% CMC Carboxymethyl cellulose	---	---	---
Standard (Piperazine citrate)	10 mg/ml	4.57±0.35	17.29±0.38
Chloroform extract	10 mg/ml	52.62±0.38	72.53±0.93
	25 mg/ml	45.28±0.39	62.82±0.59
	50 mg/ml	36.85±0.78	47.57±0.38
Methanolic extract	10 mg/ml	28.15±0.28	42.26±0.32
	25 mg/ml	18.52±0.41	37.12±0.48
	50 mg/ml	13.46±0.42	26.64±0.24
Hydroalcoholic extract	10 mg/ml	42.65±0.68	52.77±0.54
	25 mg/ml	29.27±0.28	39.51±0.63
	50 mg/ml	22.81±0.21	32.22±0.37
Aqueous extract	10 mg/ml	49.43±0.38	69.52±0.92
	25 mg/ml	40.19±0.39	58.82±0.59
	50 mg/ml	31.64±0.79	46.57±0.36

All values represent Mean±SEM; n=6 in each group. Comparisons made between standard versus treated groups, $p<0.05$ was considered statistically significant. SEM: Standard error of the mean.

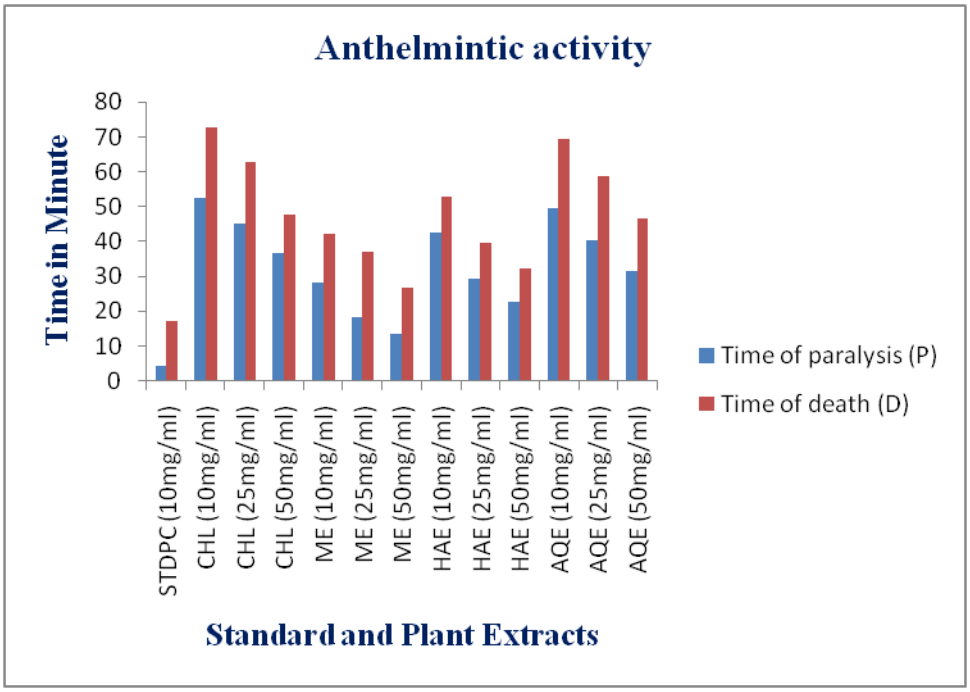

Fig. 2: Anthelmintic activity of *Albizia lebbeck* (L.) Benth. bark extracts on *Ascaridia galli*.
Where, STDPC - Standard Piperazine citrate, CHL- Chloroform extract, ME - Methanolic extract, HAE - Hydroalcoholic extract, AQE - Aqueous extract

Preliminary phytochemical screening of chloroform, methanolic, aqueous, and hydroalcoholic extract of bark of medicinal plant *Albizia lebbeck* (L.) Benth. was revealed the presence of glycosides, alkaloids, saponins, flavonoids, and tannins. As shown in Tables 1 and 2, methanolic extract exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration, for *Pheretima posthuma* and *Ascaridia galli* worms. The chloroform, 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 medicinal plant *Albizia lebbeck* (L.) Benth. showed time of paralysis is 9.26 min. and time of death is 25.44 min. and hydroalcoholic

extract revealed that the time of paralysis is 15.68 min. and time of death is 28.13 min., aqueous extract revealed that time of paralysis is 30.04 min. and time of death is 42.78 min., while chloroform extract showed time of paralysis is 35.13 min. and time of death is 45.97 min. respectively, against *Pheretima posthuma* at 50 mg/ml concentration. The reference drug Piperazine citrate showed that the time of paralysis is 2.7 min. and time of death at concentration of 10 mg/ml is 16.11 min., respectively. The methanolic extract of the bark of medicinal plant *Albizia lebbeck* (L.) Benth. showed time of paralysis is 13.46 min. and time of death is 26.64 min. and hydroalcoholic extract revealed time of paralysis of 22.81 min. and time of death 32.22 min., aqueous extract revealed time of paralysis of 31.64 min. and time of death 46.57 min., while chloroform extract revealed time of paralysis of 36.85 min. and time of death 47.57 min. respectively, against *Ascaridia galli* at 50 mg/ml concentration. The reference drug Piperazine citrate showed the paralysis at 4.57 min. and time of death at 10 mg/ml conc. 17.29 min., respectively.

Piperazine citrate by increasing chloride ion conductance in worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.[28] The extracts of the bark of medicinal plant *Albizia lebbeck* (L.) Benth. 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 chemicals constituent within them. The chemical constituent tannins were shown to produce anthelmintic activities.[29] Chemically, tannins are polyphenolic compounds.[30] Some synthetic phenolic anthelmintics, for example, albendazole, niclosamide, oxyclozanide, bithionol, etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation.[31] It is possible that tannins contained in the extracts of the bark of medicinal plant *Albizia lebbeck* (L.) Benth. produced similar effects. Another possible anthelmintic effect of tannins is that they can be bind to free proteins in the gastrointestinal tracts of host animal[32] or glycoprotein on the cuticle of the parasite[33] and may cause death.

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 (Figs. 1 and 2).

CONCLUSION

The results of the present study clearly indicated that the methanolic extract of plant *Albizia lebbeck* (L.) Benth. produces anthelmintic activity against Indian earthworm *Pheretima posthuma* and *Ascaridia galli.* The plant possesses significant anthelmintic activity at 50 mg/ml concentration measured by time taken for paralyze and death of the earthworms. The current investigation leads to conclusion that the bark of plant *Albizia lebbeck* (L.) Benth. has potent anthelmintic activity when compared with the conventionally used drug. Further studies can be explore using *in vivo* models and to carry isolation of active constituents from methanolic extract and establishment of the effectiveness and pharmacological rationale for the use of plant *Albizia lebbeck* (L.) Benth. as an anthelmintic drug.

ACKNOWLEDGMENT

The corresponding author is very grateful to Principal Dr. Rajendra D. Wagh, DCS's A.R.A. College of Pharmacy, Nagaon, Dhule, for providing necessary facilities, support and proper guidance throughout the research work.

AUTHOR'S CONTRIBUTIONS

We declare that this work was done by the corresponding author and co-author named in this article. Dr. Umesh Prabhakar Joshi and Dr. Kailaspati Prabhakar Chittam collected the data and statistically analyzed the data by Mr. Chetan Vijay Jain and Mr. Sayyed Tahir Farid. Dr. Umesh Prabhakar Joshi and Dr. Kailaspati Prabhakar Chittam who make proof read the whole manuscript and make the necessary changes and helped in developing the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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