



**EVALUATION OF *IN VITRO* ANTHELMINTICS STUDY OF LEAF EXTRACT
ALBIZIALEBBECK AGAINST *PHERETIMA***

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ABSTRACT

Control of helminth parasites is a key challenge for human and veterinary medicine. In the absence of effective vaccines and adequate sanitation, prophylaxis and treatment commonly rely upon anthelmintics. There are concerns about the development of drug resistance, side-effects, lack of efficacy and cost-effectiveness that drive the need for new classes of anthelmintics. Despite this need, only three new drug classes have reached the animal market since 2000 and no new classes of anthelmintic have been approved for human use. So where are all the anthelmintics? What are the barriers to anthelmintic discovery, and what emerging opportunities can be used to address this? This was a discussion group focus at the 2019 8th Consortium for Anthelmintic Resistance and Susceptibility (CARS) in Wisconsin, USA. Here we report the findings of the group in the broader context of the human and veterinary anthelmintic discovery pipeline, highlighting challenges unique to antiparasitic drug discovery. We comment on why the development of novel anthelmintics has been so rare. Further, we discuss potential opportunities for drug development moving into the 21st Century.

KEYWORDS: Anthelmintic activity, Earthworm, Pheretima posthuman, *Albizia lebeck* etc.

INTRODUCTION

Anthelmintics are drugs that are used to treat infections caused by parasitic worms, also known as helminthes. These drugs work by either killing the worms or preventing them from reproducing and spreading throughout the host's body. Helminthic infections are among the commonest infections in man, affecting a large proportion of the world's population. In developing countries they pose a major threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia. Anthelmintics are drugs that either kill or expel infesting helminths and the gastrointestinal tract is the abode of many helminths, although some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal but is a major cause of morbidity.^[1]

Helminthiasis is a macro parasitic disease observed in humans and animals in which a part of the body is infested with parasitic worms such as Roundworms [Nematodes], Tapeworms [Cestodes] or Flukes [Trematodes]. Typically the worm's reside in the GI Tract. Anthelmintics are drugs that destroy or expel

parasitic intestinal worms from the body, by either vermifuges [stunning] or vermicides [killing]. Most of the existing anthelmintic produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea. Since ancient times herbal drugs are used for the treatment of parasitic diseases in a human without any side effects. To eradicate the side effects of the present allopathic drugs now scientist are moving towards the herbal drugs what our ancient peoples used.

The WHO [World Health Organization] estimated that 80% of population in developed countries rely on traditional medicine mostly plant drugs for their Primary Health Care needs. It is estimated that by the year 2025 about 57% of population in developing countries will be influenced by this infection. The gastrointestinal helminthes become resistant to currently available anthelmintic drugs, therefore, there is a foremost problem in the treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintic.^[2] In recent years, there has been growing interest in developing new anthelmintic drugs from natural sources. One such source is *Albizia lebeck*, a tree native to India and Southeast Asia. The leaves of this tree have been traditionally used in India Ayurvedic

medicine to treat various ailments, including parasitic infections. Given this traditional use, it is possible that *Albizia lebbbeck* could be a source of novel anthelmintic compounds.

Albizia lebbbeck (L.) Wild is commonly known as 'Shrisha'. It is found throughout India ascending to 900m in the Himalayas and also in the Andaman. Bark is dark brown to greenish black, rough with longitudinal and transverse fissures on outer surface inner surface whitish with fine longitudinal stations. The sapwood is white or yellowish white and the heartwood is dark brown, streaked with dark and white shades. Leaves are bipinnate with 8-18 leaflets. Flowers are stalked, greenish yellow. Flowering and fruiting season starts from April to June. Pods are yellowish brown with 6-10 seeds. Mature pods remain on the tree for long period

and are available till May-July. The free is good substitute for teak and. Leaf of *A. lebbbeck* has great medicinal as well as nutritional value. Phytochemically the leaves contain saponin, tannin and cardiac glycoside.

In Ayurveda, expressed juice of leaf is advocated to instill in to nostrils in case of poisoning. The plant has been reported to possess anti-inflammatory. Anti-allergic activity of hot aqueous stem bark decoction and its butanoic fraction in various models like anti PCA or mast cell stabilizing activity anti-convulsion effect antioxidant. Systematic pharmacogenetic and phytochemical studies have been reported till date. So a detailed investigation of fresh as well as powdered of leaves of *Albizia lebbbeck* L. (Wild) has been carried out using various pharmacogenetic and physico- chemical parameters.^[3]



Fig. 01: *Albizia lebbbeck*. [tree, seeds, pods, flower]

Binomial name: *Albizia lebbbeck* (L.) Benth.

Synonyms

Albizia lebbbeck (L.) Benth. (Family – Fabaceae) is generally known as Lebbek Tree, siristree in English; shiris Tree, siris in Hindi, shirish tree in gujrati, siris tree in urdu, Bengali and marathi, bagemara, bagey, hombage in kannad, sitapuspa, sukapriya tree, bhanditree, mrdupuspa tree in sanskit, nanmenivaka, vaka in malyalam, sirisha in oriya vakai in tamil, sareehn and sirish in Punjabi and dirisena in teulgu.

Common names

East Indian walnut, fry wood, koko, lingua de mulher, lebbek, lebbek tree, siris tree, coração de negro, women's tongue tree, lebbekboom, ebano oriental, lingua de sogra.

Taxonomical Classification

- Domain: Eukaryote
- Kingdom : Plantae
- Division: Magnoliophyta
- Class: M Fabaceae.

Distribution

Albizia lebbbeck (L.) Benth. is a species of *Albizia*. The word *Albizia* was used by Italian Naturalist in 18th century (Chopra *et al.*, 1984). The species is native to Indonesia, India Myanmar, Andaman Island, and Tropical Africa, Northern Australia, Bangladesh, Malaysia, Nepal, Pakistan, Thailand country and Asia and also distributed in subtropical and tropical areas The genus has about 150 species and it is commonly distributed in central - South America and Africa. Plants of *Albizia* genus are fastly growing in the tropical and

sub tropical parts of India as well in the world.^[4] These trees distributed in Rajasthan state.^[5]

Description of Plant

Albizia lebbek (L.) Benth. is the tree of the medium length and deciduous tree.^[6] The stem is full of the secondly growth and they have the large brown bark and the typical of the Mimosaceae features and the physiological features. The leaves are bipinnate, 7.515cm long, with one to four pairs of pinnae, each pinna with 6–18 leaflets. Seeds the Mimosaceae features and the physiological features. The leaves are bipinnate, 7.515cm long, with one to four pairs of pinnae, each pinna with 6–18 leaflets. Seeds.

Properties and actions^[7]

Guna: Ruksha, Teekshna, Laghu
Rasa: Madhura, Tikta, Kashaya
Vipaka: Katu

PHYTOCONSTITUENTS FOUND IN THE PLANT

It consists of various Phytochemicals, including major alkaloids, flavonoids, Saponins, terpenoids, Phenols, tannins and proteins Stem bark yields 7 to 11% tannins; D-catechin D-leucocyanidin and it yield seven compounds including friedlan-3-one and γ -sitosterol. Three Saponins were isolated from bark. Oil obtained from seeds contains sterols, methyl sterols, triterpene alcohol, tocopherol, hydrocarbons and carotenoids, cycloeucaenol, 24-ethylphenol, cycloartenol. Flowers yield triterpenoids, saponins, labbekanin D and 4, glycosides, lebbekannins D, F, G, and H. High concentration of non-protein amino acids is found in young a leaf that is uncommon to other plants. The leaves contain echinocystic acid and it yielded flavon, vicenin II and β -sitosterol. Mature leaves of *A. lebbek* contained keto acids including phosphoenolpyruvate glyoxylate, oxaloacetate, and α -oxoglutarate; vicenin-2, reynoutrin, rutin, myricitrin, and robinin. Leaves also have alkaloids, flavonoids, tannins, saponins, carbohydrates. Seeds contain glycosides, proteins/aminocids (arginine and lysine), resins, reducing sugars, saponins flavonoids, glucosides. while aminoacids like glutamic acid and aspartic acid are present in the highest concentrations in pods. Linoleic acid was detected as the major fatty acid in pod and seed oil.^[8]

Therapeutic Uses^[9]

A. lebbek (L.) Benth. is the plant of the great medicinal uses and they are used for the timber as well as the medicinal point of view, in the Indian medicines system, in many kinds of the disorders of the system the plants parts and there extracts has been used as the ayurvedic, siddha medicinal treatment, well a number of the successful medicines has been formed which are used global for the treatment of the diseases. The timber of the wood is also very useful and they are used for the formation of the furnitures and the other things. The roots of the rhizobium are not the nitrogen fixators but

the presence of the nodules in the system sometimes produces the elicitors and the products in the nature.

1. Bark

Mildly thermo genic, astringent, acrid, bitter, sweet, expectorant, aphrodisiac, depurative, ophthalmic, tonic, restorative diseases of the gum and toothache, weakness, alexiteric, cures diseases of blood, anthelmintic, itching, leucoderma, excessive perspiration, skin disease, piles, deafness, scabies, syphilis and boils helminthes infections, bronchitis, dental infections, leprosy, antidiarrheal activity and paralysis pruritus, eczema, paralysis and worm infestation.

2. Flower

Asthma, tropical pulmonary eosinophilia bronchitis and chronic cough, chronic catarrh, inflammation, poisoning, scrofula, seminal weakness, skin diseases, ophthalmopathy, leucoderma and leprosy.

3. Leaves

Antiseptic, anti-cancer activity, anti-tubercular, tuberculosis, antimicrobial, anti-ovulatory, reddishness of eyes, and trauma, anti-protozoal, anti-dysenteric and anti-fertility.

4. Seed

The seed oil is applied topically to cure leucoderma, scrofulous swellings and astringent, aphrodisiac, diarrhoea and piles.

5. Heartwood

Making cabinet timber and also in the doors of Chinese temples, veneer and furniture.

In Vitro Studies

In vitro studies refer to experiments that are conducted outside of a living organism, usually in a test tube or petri dish. These studies are commonly used in anthelmintic research to evaluate the efficacy of potential compounds against parasitic worms. In vitro studies can provide valuable information about the mechanism of action of anthelmintics and their potential toxicity. To conduct an in vitro study, researchers typically isolate the target parasite and expose it to the test compound. The researchers then observe the effects of the compound on the parasite, such as changes in motility or viability. The present study was carried out to evaluate the anthelmintic potential of *Albizia lebbek* against *Pheretima posthuman*. The study will help in developing easily available anthelmintic drug with minor side effects to support the livestock industry. The rationale behind studying *Albizia lebbek* as source of anthelmintic compounds is due to the increasing problem of drug resistance in current anthelmintics. The rationale behind studying *Albizia lebbek* as source of anthelmintic compounds is due to the increasing problem of drug resistance in current anthelmintics.

MATERIALS AND METHODS

MATERIALS

Plant selected

In the present study, *Albizia lebbek* was selected because of its therapeutic uses, the part used was leaves.

Chemicals and reagents used

- Ethanol
- Methanol
- Normal saline water
- Benedict's reagent
- Biuret reagent (NaOH & CuSO₄)
- Ferric chloride solution
- Wagner's reagent.

Equipment's used

- Stoppered glass container
- Beaker
- Petri dish
- Water bath
- Measuring cylinder
- Glass rod
- Test tubes.

Drugs used: Albendazole.

Animal used: *Pheretima posthuman*. (Indian adult earth worms)

METHODS

Collection and Authentication of plant methods:

Albizia lebbek leaves were collected in the month of August 2023 from Koratagere village, Dist of Tumkur, in the state of Karnataka, India. The plant was authenticated by Range forest officer Koratagere, Tumkur district. The

fresh leaves were collected, removed all the dirty matter, washed, dried under sun light and powdered by using mixer grinder.



Figure 02: *Albizia lebbek* leaves.

Preparation of plant extract

The leaves of the plant were dried under sunlight and grounded into fine powder by using mixer grinder.



Figure 03: [A] Wet leaves, [B] Dried leaves, [C] Powdered leaves: The powdered leaf of the plant was extracted by two methods namely Cold maceration and Decoction method by using ethanol, methanol & water.

Cold maceration

It was performed by using ethanol and methanol 10 gms of powdered leaves was weighed and soaked in 100ml of ethanol and 100ml of methanol, separately in a stoppered glass container for about 3 days at room

temperature with occasional stirring, until the soluble matter has dissolved. After 3 days the solution was filtered through filter paper. And the filtrate thus obtained was collected into a beaker.



Figure 04: Cold maceration extraction method.

Decoction method

In this method, 10g of powdered leaves was weighed, added 100ml of distilled water and 100ml of ethanol separately in a beaker, then boiled the mixture in a specified volume of water in a water bath for a defined time period, and then it is cooled and filtered by the filter paper. The filtrate obtained was collected into another beaker.

Test for carbohydrates

Benedict's test: Equal volume of benedict's reagent and the test solution extract was mixed, heated in a water bath for 5 mins, gives greenish yellow precipitate that confirms the presence of carbohydrates in the Ethanolic extract of *Albizia lebbek* leaves.

Test for alkaloids**Test For protein Biuret test**

3 ml of extract and 4% of NaOH placed in a test tube followed by the addition of 5 drops of CuSO_4 solution, results in the formation of violet color indicates the presence of proteins in the extract.

Test for tannins-Ferric chloride test

2ml of extract followed by the addition of 5% ferric chloride solution, results in the formation of a blue color indicates the presence of tannins.



Figure 05: Decoction extraction method.

Test for Saponins: Foam test

Shake the drug extract vigorously with water, in a test tube. Persistent foam is not formed, indicates the absence of tannins in the alcoholic extract of *Albizia lebbek*.

Phytochemical Screening

The alcoholic extract (Methanolic and Ethanolic) of powder of dried *Albizia lebbek* leaves were subjected for the following chemical tests for the identification of various active constituents.



Figure 06: Phytochemical investigation.

Table 01: Phytochemical test for alcoholic extract of powder of dried *Albizia lebbbeck* leaves.

Sr.no.	Material	Functional groups	Reagent	Observation	Result
1	Alcoholic extract of Powder of dried <i>Albizia lebbbeck</i> leaves	Carbohydrates	Benedict's reagent	Greenish yellow ppt	Present
2		Alkaloids	Wagner's reagent	Reddish brown ppt	Present
3		Proteins	Biuret reagent	Violet color	Present
4		Tannins	Ferric chloride Solution	Blue color	Present
5		Saponins	Water	Foam is not Formed	Absent

The Phytochemical screening of alcoholic extract of *A. lebbbeck* showed the presence of carbohydrates, proteins, alkaloids, tannins.

Collection of Worms

Indian Adult Earthworms (*Pheretima postuma*) were used to evaluate the anthelmintic activity of plant extract due to its anatomical and physiological resemblance with intestinal round worm parasites present in human beings and also due to their abundant and easy availability. The worms were collected from damp soil and from manure, and then they were washed with normal saline water in order to remove all kinds of dirt and faecal matters.



Figure 07: Earthworms.

The anthelmintic activity was carried according to the standard method. Adult Indian earthworm *P. postuma* has anatomical and physiological resemblance to the intestinal round worm parasites of human beings. Fifty five Indian adult earthworms were collected and groups were divided into eleven containing five earthworms in each group. Each group was treated with any one of 10 ml of standard drug Albendazole (20mg/ml, 30mg/ml); Methanolic extract of *A. lebbbeck* (10mg/ml, 20mg/ml, 30mg/ml) and Aqueous extract of *A. lebbbeck* (10mg/ml, 20mg/ml, 30mg/ml) in eleven different petridishes. The earthworms were placed in the petridishes containing the different concentrations of ethanolic, methanolic, aqueous extract of *A. lebbbeck* and the Std. drug Albendazole. Each Time of paralysis recorded when no movement of any sort observed except when shaken vigorously. Time of death was recorded when the worms neither moved when shaken. Finally, the test results were compared with Std reference compound Albendazole.

RESULTS

Anthelmintic activity of ethanolic, methanolic, aqueous extract of *A. lebbbeck* and standard Albendazole.

Evaluation of *In vitro* Anthelmintic activity

Extracts/Drug(Treatment)	Concentrations(mg/ml)	Time Taken for paralysis(min)	Time taken for death(min)
Standard drug(Albendazole)	20 mg/ml	350	410
	30 mg/ml	245	305
Ethanolic extract of <i>A. lebbbeck</i>	10 mg/ml	2 ± 3	2 ± 3
	20 mg/ml	1 ± 1.5	1 ± 2
	30 mg/ml	0.5 ± 1	1 ± 1.5
Methanolic extract of <i>A. lebbbeck</i>	10 mg/ml	3 ± 4	4 ± 5
	20 mg/ml	1 ± 2	3 ± 4
	30 mg/ml	1 ± 1.5	1 ± 2
Aqueous extract of <i>A. lebbbeck</i>	10 mg/ml	17 ± 18	30 ± 31
	20 mg/ml	15 ± 16	30 ± 30.5
	30 mg/ml	14 ± 15	25 ± 26



[A] Ethanolic extract [B] Methanolic extract [C] Standard Drug [D] Aqueous extract
Figure 08: *In vitro* experimental model setup to evaluate the anthelmintic activity.

Ethanolic leaf extract of *A. lebeck* shows significant effect on *Pheretima posthuma* higher concentrations of extracts produce a paralytic effect much easier and time taken for death was shorter. It shows the maximum efficacy at 30mg/ml, than the standard drug Albendazole.

DISCUSSION

Posthuman has been used in the screening of anthelmintic agents, because of their resemblance to intestinal round worms in reaction to Anthelmintics and due to their assay availability in this assay we have evaluated the anthelmintic activity of three different extract of *A. lebeck*. The results have been summarized in Table no.2. It is seen that all the extracts in their different concentrations namely; 10mg/ml, 20mg/ml and 100mg/ml showed anthelmintic activity comparable with the standard drug Albendazole in their respective concentration; 20mg/ml and 30 mg/ml. All the extracts showed a dose dependent increase in the anthelmintic action taking shortest time for paralysis and death for Ethanolic extracts and *Albizzia lebeck* showed the highest activity followed by aqueous and Methanolic extracts, At the concentration of 30mg/ml, the ethanolic extract of *A.lebeck* showed a remarkable anthelmintic activity which was even greater then the standard drug.

Albendazole at the same concentration with time of paralysis and death of the worms less of the former than the latter. Therefore, from this study we can presume that out of the many phytoconstituents present, one or few are responsible for the exhibited anthelmintic activity.

CONCLUSION

It can be concluded that the ethanol leaf extract of *Albizzia lebeck* produces better anthelmintic activity against Indian earthworm *Pheretima posthuman* than compared to aqueous extract of *Albizzia lebeck* and standard drug Albendazole. At higher concentrations,

the Ethanolic extract showed higher activity. From the biological assay performed and observations noted, it can be concluded that *Albizzia lebeck*, Benth (Fabaceae), possesses significant anthelmintic activity when compared with the normally used drug. And hence it suggests that this medicinal plant is worthy to be investigated as an effort to yield a new anthelmintic agent.

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