



## PRELIMINARY PHYTOCHEMICAL SCREENING AND IN VITRO ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAF RACHIS OF *AZADIRACHTA INDICA* Linn. (NEEM)

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### ABSTRACT:

The aim of the present study is to evaluate anthelmintic activity of crude ethanolic extract of leaf rachis of *Azadirachta indica* linn. (neem) against *Pheretima posthuma* as test worm. Four concentrations (10 mg/mL, 15 mg/mL, 25 mg/mL, 50 mg/mL) of ethanolic extract were studied in the activity, which involved the determination of the time taken for paralysis (P) and the time taken for death (D) of the worms. Albendazole (10 mg/mL) was included as standard reference and saline water as control. The results of present study indicated that crude ethanolic extract exhibited significant anthelmintic activity at concentration 50 mg/mL compared to reference Albendazole. The anthelmintic activity of ethanolic extract of Leaf Rachis of *Azadirachta indica* linn therefore being demonstrated for the first time and showed the best anthelmintic activity.

**Key words:** *Azadirachta indica* linn (Neem), *Pheretima posthuma*, Anthelmintic activity, Albendazole, Ethanolic extract.

### 1. INTRODUCTION:

Helminthiasis is the most important animal diseases amongst the tropical people. The disease is highly prevalent in the third world countries [1]. The increasing problems of helminthic infections have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants provide a source of botanical anthelmintics [2]. A number of medicinal plants have been used to treat parasitic infections in man and animals [3]. The plant *Azadirachta indica* (neem) is well known in India and its neighbouring countries as one of the most important medicinal plant having a wide range of biological activity. The plant belongs to the family Meliaceae [4,5]. The common names of *Azadirachta indica* are Arabic- neeb, Assamese-neem, Bengali-nim, English-neem

tree, French-azadirac del'Inde, margosier, German-indischer zedrach, Gujarati- limbda, dhanujhada, Hindi-neem, Kannada-bevu, Malayalam-aryaveppu, Manipuri-neem, Marathi-kadunimba, Nepal-neem, Odiya-neem, Punjabi-nimmi, Sanskrit-arishtha, pakvakrita, nimbaka, Tamil-veppai, Telugu-vepa and Urdu-neem.

The plant is traditionally used and possesses antibacterial, antifungal, antiviral, antihistamine, anthelmintic and antiseptic properties. The neem leaves, flowers, seeds, roots, bark and fruits are utilized to treat inflammation, infections, skin diseases etc. [6].

The neem tree has pharmaceutical and pesticide controlling qualities. Neem is also useful to control diarrhoea. The plant is also

possesses analgesic activity. Neem extract possess anti-diabetic & anti-viral properties. The bark is useful in treating malarial fever. It also possesses antidandruff property.

According to the literature survey, there is no systematic study on leaf Rachis of *Azadirachta indica* (neem) has been reported for its anthelmintic activity. So the present study was carried out to evaluate the in vitro anthelmintic activity of crude ethanolic extract of leaf rachis of *Azadirachta indica* (neem) against *Pheretima Posthuma*.

## 2. MATERIALS AND METHODS

### 2.1 Collection of plant materials:

The fresh plant of *Azadirachta indica* (neem) was collected in the Aditya College of Pharmacy premises situated at Surampalem, East Godavari (Dist.), AP, India. The plant was authenticated by Department of Pharmacognosy, Aditya College of Pharmacy. The fresh Leaf Rachis were collected and shade dried under normal environmental conditions, powdered, stored in a closed container.

### 2.2 Preparation of extract:

The powdered Leaf Rachis were extracted with ethanol using maceration method. The extract was filtered and concentrated by distilling of the solvent to obtain crude extract. The extract obtained was then evaporated to dryness under reduced pressure.

### 2.3 Phytochemical screening:

Qualitative chemical tests were carried out on the ethanolic extract of Leaf Rachis of *Azadirachta indica* for the determination of phytochemical constituents as per the standard procedure [7,8].

## 2.4 Biological study:

Healthy adult Indian earthworms *pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings were used in the present study [9-11] for evaluating anthelmintic activity. All earthworms were of approximately equal size. They were collected from local moist place washed and kept in water.

## 2.5 Drugs and chemicals:

Albendazole, ethanol and other chemicals are of analytical grade were procured from different suppliers and used during the experiment.

## 2.6 Anthelmintic activity:

The anthelmintic activity was carried as per the method of Ajaiyeoba et al., [12] with necessary modifications. Indian adult earthworms collected from moist soil and washed with normal saline solution to remove all faecal matter were used for anthelmintic activity. Different concentrations of the dried extract [10, 15, 25 and 50 mg/mL in saline solution with tween 80] were prepared. 25 ml of each concentration of ethanolic extract was delivered into a petri dish. Then six worms (same type) were placed in it. Observations were made for the time taken for paralysis and death of individual worms. Time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time taken for the death of worms was also recorded when the worms neither moved when shaken vigorously nor when dipped in warm water [50° C] followed with fading away of their body colours. Albendazole [10 mg/mL in vehicle tween 80] was used as standard and saline solution was used as control.

### 3. RESULTS AND DISCUSSION

The results of phytochemical screening were shown in Table 1.

The results of anthelmintic activity were shown in Table 2.

**Table 1: Phytochemical constituents present in the ethanolic extract of Leaf rachis of *Azadirachta indica* (Neem)**

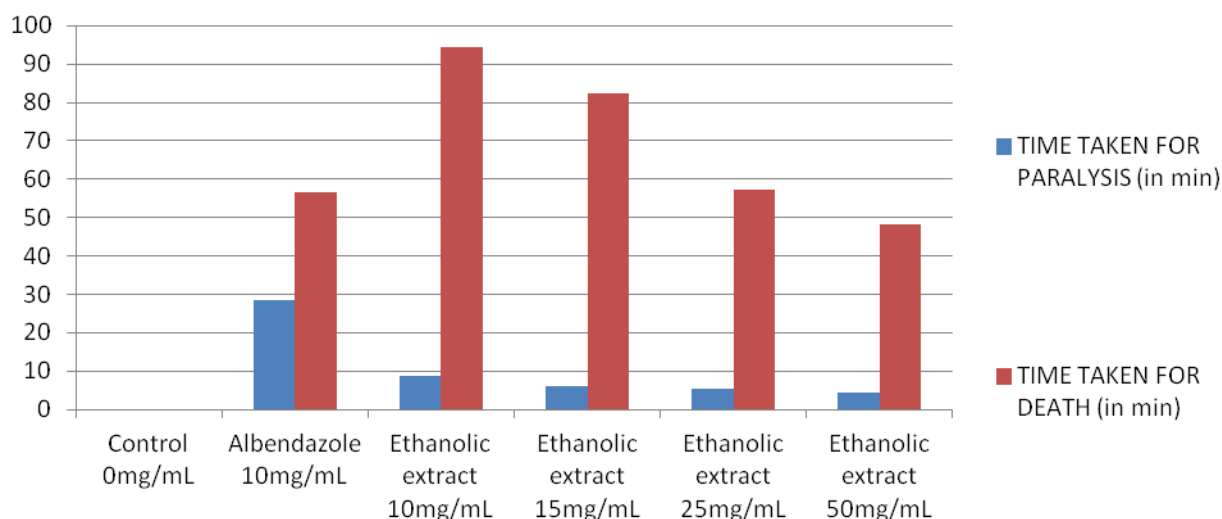
Phytochemical	Test	Observation	Result
Alkaloids	Hager's test	Yellow precipitate	Positive
Amino acids	Millon's test	No white ppt	Negative
Carbohydrates	Thymol test	Purple colour	Positive
Flavonoids	Sodium hydroxide test	Yellow to colourless	Positive
Cardiac glycosides	Baljet test	No yellow to orange colour	Negative
Steroids	Salkowski's test	Yellow colour ring to red colour	Positive
Tannins	Ferric chloride test	Green colour	Positive

**Table 2: *In vitro* anthelmintic activity of ethanolic extract of Leaf rachis of *Azadirachta indica* (Neem) against earthworms (*Pheretima Posthuma*)**

S.No.	Sample	Concentration (mg/mL)	Time taken for paralysis (in min)	Time taken for death (in min)
1.	Control	0	0	0
2.	Albendazole	10	28±0.37	56±0.62
3.	Ethanolic extract	10	8±0.61	94±0.41
4.	Ethanolic extract	15	6±0.22	82±0.33
5.	Ethanolic extract	25	5±0.25	57±0.22
6.	Ethanolic extract	50	4±0.46	48±0.21

The ethanolic extract of Leaf Rachis of *Azadirachta indica* exhibited anthelmintic activity in a dose dependent manner. The ethanolic extract at dose 50 mg/mL caused paralysis in 4 minutes and death in 48 minutes against *pheretima posthuma* compared to the reference standard Albendazole. Considering time taken for paralysis and death of earthworms, the ethanolic extract [50mg/ml] was more effective than the standard Albendazole (10 mg/mL). Similarly the ethanolic extract at concentrations [10, 15, 25 mg/mL] also exhibited anthelmintic activity compared to

that of standard Albendazole [10 mg/mL] and was represented by statistically via bar diagram as shown in Fig. 1. The ethanolic extract of different concentrations of Leaf Rachis of *Azadirachta indica* not only demonstrated paralysis but also cause death in shorter time as compared to reference drug Albendazole. The study on the anthelmintic activity of the Leaf Rachis of *Azadirachta indica* was not available till now. From this study, it may be concluded that the ethanolic extract Leaf Rachis of *Azadirachta indica* possess potent anthelmintic activity.



**Fig. 1: *In vitro* anthelmintic activity of ethanol extract of Leaf Rachis of *Azadirachta indica* against earthworms (*Pheretima Posthuma*)**

#### 4. CONCLUSION

The phytochemical studies indicated the presence of several types of chemical constituents. It could be concluded that the ethanol extract of Leaf Rachis of *Azadirachta indica* possess good anthelmintic activity when compared to standard drug Albendazole. Further, it would be interesting to isolate the actual responsible phyto constituents that are present in the crude extract for anthelmintic activity.

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