

INVESTIGATION OF IN VITRO ANTHELMINTIC ACTIVITY OF AERIAL PARTS OF LEUCAENA LEUCOCEPHALA

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ABSTRACT

Leucaena leucocephala belongs to the family Fabaceae is a small tree originated in Mexico and is naturalized throughout the tropical areas. The present study was undertaken to evaluate anthelmintic activity of 50% hydroethanolic extract of aerial parts (leaves, seeds, stem) of Leucaena leucocephala through invitro assay using Pheretima posthuma as test worms. Different concentrations of plant extracts were tested and results were expressed in terms of time for paralysis and death of worms. Albendazole was used as a standard and carboxy methyl cellulose (CMC) was used as a control. All the extracts were able to paralyse and kill the earthworms. The result obtained in the study led to the conclusion that leaves of Leucaena leucocephala showed a significant anthelmintic activity at a highest concentration of 100mg/ml followed by seed and stem.

KEYWORDS: Leucaena leucocephala, anthelmintic, Pheretima posthuma, paralysis, death.**INTRODUCTION**

Leucaena leucocephala is a flowering plant commonly referred as lead tree.^[1] In India, it is popularly called as kubabul or subabul.^[2] During 1970s and 1980s it was promoted as a "miracle tree" due to its broad spectrum applications in various fields.^[3] The aerial parts of Leucaena leucocephala has reported to have different medicinal uses which include antioxidant, antidiabetic, contraceptive, abortive and nutritive activity.^[4,5,6] It is also used in the treatment of measles, scurf and remedy for diabetes.^[7] The plant is selected based on easy availability and posses various medicinal properties and not exploited for anthelmintic activity.^[7,8]

Helminthes infections are now being recognized as a cause of chronic ill health. Helminthiasis is one of the major prevalent diseases in the world particularly in tropical countries which is resulting from worm infestation.^[9] In developing countries they raise a large threat to public health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia.^[10] The helminths parasites are mainly present in intestinal tract of human body and they are also found in tissue as their larvae migrate towards them.^[11]

Helminthiasis is one of the common diseases of all ages and the body is infested with worm such as pinworm, roundworm or tapeworm.^[12] The infected people excrete helminth eggs in their faeces which then contaminate the soil in areas with inadequate sanitation^[13] and other

people can be infected by ingesting eggs or larvae in the soil.^[14] The gastrointestinal helminths become resistant to currently available anthelmintic drug which is the foremost problem in the treatment of helminths diseases.^[15] As per WHO, synthetic drugs are commonly used in the treatment of helminth infestation in human beings but these drugs are out of reach of millions of people and have a lot of side effects. In view of this, an attempt has been made to study the anthelmintic activity of herbal drugs.

MATERIALS AND METHODS**Plant material**

The aerial parts (leaves, seeds, stem) of Leucaena leucocephala were collected in fresh condition without any physical defect from Coimbatore, Tamil Nadu. They were washed thoroughly with distilled water and air dried in shade, powdered using a blender and polythene covers at room temperature for further analyses.

Preparation of plant extract

10 g of powdered plant samples (leaves, seeds, stem) were subjected to cold extraction using 50% hydroethanolic extract (100 ml) with occasional stirring for 3days. After 3 days, the extracts were filtered using Whatmann No.1 filter paper and were evaporated to dryness at low temperature (<40°C) under reduced pressure in a rotary vacuum evaporator. The powders obtained were stored in a refrigerator and were used for the further analyses.

Worm collection

Earthworms, *Pheretima posthuma* have been used widely for the investigation of in vitro anthelmintic activity. Indian adult earthworms of average size 6-8cm were collected from water logged areas of soils and cleaned thoroughly with normal saline to remove all filthy matter and were evaluated for anthelmintic activity.

Anthelmintic Activity

The anthelmintic activity was evaluated according to the method of Ghosh (2009).^[16] The adult earthworms (*Pheretima posthuma*) were used in the present study because they resemble anatomical and physiological characteristics with the intestinal roundworm parasite of human being. Earth worms were placed in petriplates containing 50% hydroethanolic extracts of aerial parts at various concentrations (50, 100mg/ml). Each petriplate was placed with 2 worms and observed for paralysis and death. Paralysis was noted when no movement could be observed except when the worms were shaken strongly. Time of death for individual earthworms was recorded when the worms showed no movement when neither

shaken nor given external stimuli. The test results were compared with the standard drug albendazole.

RESULTS

From table 1 it is evident that 50% hydroethanolic extract of *Leucaena leucocephala* at concentrations of 50 and 100mg possess anthelmintic activity. The leaves, seed and stem showed paralysis at 33.33±0.74, 44.96±0.66 and 53.13±0.72 minutes respectively and death at 70.16±0.96, 92.36±0.50 and 108.06±0.80 minutes respectively. The standard drug albendazole showed paralysis at 22.22±0.90 and death at 42.82±0.37 minutes. The time taken for paralysis / death of worms was observed to be inversely proportional to the activity of the extracts. The anthelmintic effect of the extracts is comparable to that of the standard drug albendazole. In control, no paralysis or death was observed after 24 hours. Thus from the observations it is clear that the leaves extract of *Leucaena leucocephala* was found to have more anthelmintic activity.

Table 1: Anthelmintic effect of aerial parts of *Leucaena leucocephala*.

Treatment	Concentration (mg/ml)	Time taken for paralysis (minutes)	Time taken for death (minutes)
Control	-	No paralysis	No death
Albendazole (Standard)	50	28.63±1.21	56.71±0.89
	100	22.22±0.90	42.82±0.37
Leaves	50	40.40±1.17	82.53±0.90
	100	33.33±0.74	70.16±0.96
Seeds	50	51.03±0.75	101.43±1.59
	100	44.96±0.66	92.36±0.57
Stems	50	62.36±1.12	110.03±0.78
	100	53.13±0.72	108.06±0.80

The values are expressed as Mean ± SD. (n=3)

DISCUSSION

Plants have long been studied as potent source of chemicals for controlling animal and human parasites due to their numerous medicinal and therapeutic properties.^[17] Parasitic helminths affect man and animals which cause considerable hardship and stunted growth. Since tremendous advances has been made during the last decade and substantial number of synthetic precursors have been derived but unfortunately no effective medicine has been developed so far to manage the damage caused by parasite. Moreover the problems associated with the use of such drugs cause some serious side effects and development of resistance drives the severity of infection to the next level.^[18]

The phytochemical screening of *Leucaena leucocephala* revealed the presence of different secondary metabolites such as alkaloid, tannins, flavonoids, saponins, glycosides and cardiac glycosides.^[19] FT-IR and GC-MS analysis also confirmed the presence of tannins and flavonoids. The anthelmintic activity of *Leucaena leucocephala* might be due to the presence of these

phytochemical constituents which might act synergistically. It may be explained by the fact that the mortality of *Pheretima posthuma* is due to the presence of tannins which interacts with surface proteins of the worm.^[20] Another possible mechanism of tannins involved in anthelmintic activity is that, they can bind to glycoproteins on the cuticle on the parasite and can directly cause death.^[21,22] Thus the present study proved the anthelmintic activity of 50% hydroethanolic extract of aerial parts of *Leucaena leucocephala*.

CONCLUSION

The current investigation leads to the conclusion that the leaves of *Leucaena leucocephala* found to have potent anthelmintic activity when compared to other aerial parts (seed and stem). Further investigations are required to find the in vivo anthelmintic activity of *Leucaena leucocephala*.

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CONFLICT OF INTEREST

There is no conflict of interests.

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