

ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF LEUCAENA
LEUCOCEPHALAR. Jona Methusala*, Dasi Sreya Reddy¹, Dereddy Manjusha², Desam Krishna Priya³ and Ganuga Harisha⁴*(M. Pharm, Ph. D) Dept. of Pharmacology and ^{1,2,3,4}B. Pharmacy
Dr. K. V. Subba Reddy Institute of Pharmacy, Kurnool, Andhra Pradesh.

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*Corresponding Author

R. Jona Methusala

(M. Pharm, Ph. D) Dept. of
Pharmacology, Dr. K. V. Subba
Reddy Institute of Pharmacy,
Kurnool, Andhra Pradesh.

ABSTRACT

The plants play a vital role in human life. Every plant has some disease curative properties in it. Even the fodder plants have medicinal properties. In the similar way the present research was carried out to study phytochemical present in the leaves of fodder plant "leucaena leucocephala". The activity was compared with standard Tinidazole. The ethanolic extract shows significant activity when compared to the standard Tinidazole. The paralysis and death time is 15, 2, 12, 10, 5 and 30, 26, 23 minutes respectively at concentrations 10, 15 and 20 mg/ml. whereas these are 16, 13, 8 and 21, 19, 15 minutes for Tinidazole. In order to confirm the studies in vivo studies have to be conducted.

KEYWORDS

- Leucocephala
- Helminths
- Anthelmintic

INTRODUCTION: ANTIHELMINTIC ACTIVITY

The word "helminth" came from a Greek word "helmins" which means parasitic worm. Helminthiasis is a disease in which a part of the body is infested with worms such as pinworms, roundworms or tapeworms. Typically, the worms reside in the gut but may also burrow into the liver and other organs.

Leucaena leucocephala plant is a member of family "FABACEAE". It is commonly called as subabul, white popinac, white lead tree, wild tamarind. It is found in India in Himachal Pradesh and it is native to southern Mexico and northern central America. The word leucaena is derived from "Greek word leuc and caen" which refers to whitish flowers. It possesses highly nutritious values. These trees are mainly important due to their high medicinal values and they also produce firewood, timber, human food, green manure, shade and also to control erosion. Helminthic worms are highly predominant and depend on other species. These can exist as individuals or as parasites dependent on plant or animal host. In human beings helminthic infections are known as one of the most common infections. It affects a large amount of total world population. In most of the developing nations they pose a huge threat to public health and take part in the occurrence of pneumonia, anaemia, eosinophilia and malnutrition. In herbal medicine there is great relevance to the Fabaceae family of plants.

Important species among the genus leucaena which are

- L. leucocephala

- L. pallida
- L. trichodes
- L. retusa
- L. magnifica
- L. macrophylla

The leaf extracts of leucaena leucocephala contain several medicinal properties such as antihelminthic, antimicrobial, anticancer, anti-inflammatory, antioxidant, antitumor, antihistaminic, antiandrogenic, hypo-cholesteremic and hepatoprotective, antidiabetic, diuretics, antibacterial, antiproliferative, etc.,

DEFINITION

Helminths are large, multicellular organisms that are generally visible to the naked eye in their adult stages like protozoa, helminths can be either free living or parasitic in nature. The parasitic worms are a group of macro parasites encompassing a variety of species that can infect their hosts in different ways.

- Ingestion of eggs or larvae (eg: via contaminated food and water or fecal-oral route)
- Direct penetration of the skin and
- Via bite of vectors (eg: certain species of flies and mosquitoes).

CLASSIFICATION

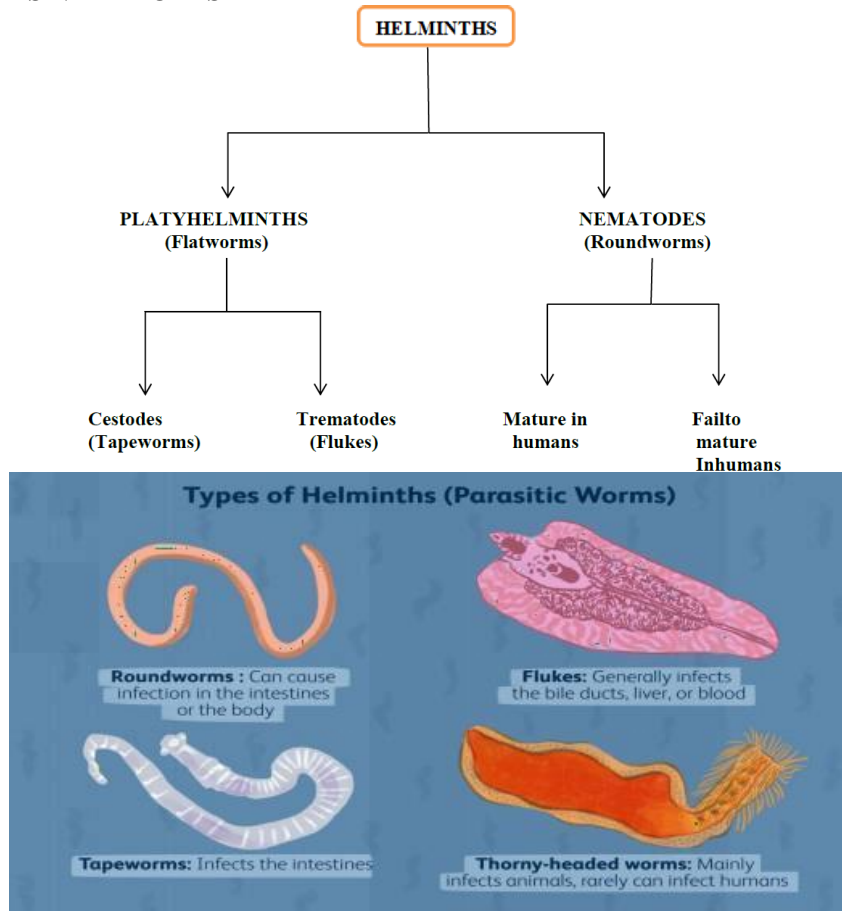
The helminths are worm-like parasites.

The clinically relevant groups are separated according to their general external shape and the host organ

they inhabit. There are both hermaphroditic and bisexual species. The definitive classification is based on the

external and internal morphology of egg, larval, and adult stages.

PLATYHELMINTHS NEMATODES



NEMATODES (ROUNDWORMS)

Nematodes are cylindrical rather than flattened; hence the common name roundworm. The body wall is

composed of an outer cuticle that has a noncellular, chemically complex structure, a thin hypodermis, and musculature.



ROUNDWORMS

CESTODES(TAPEWORMS)

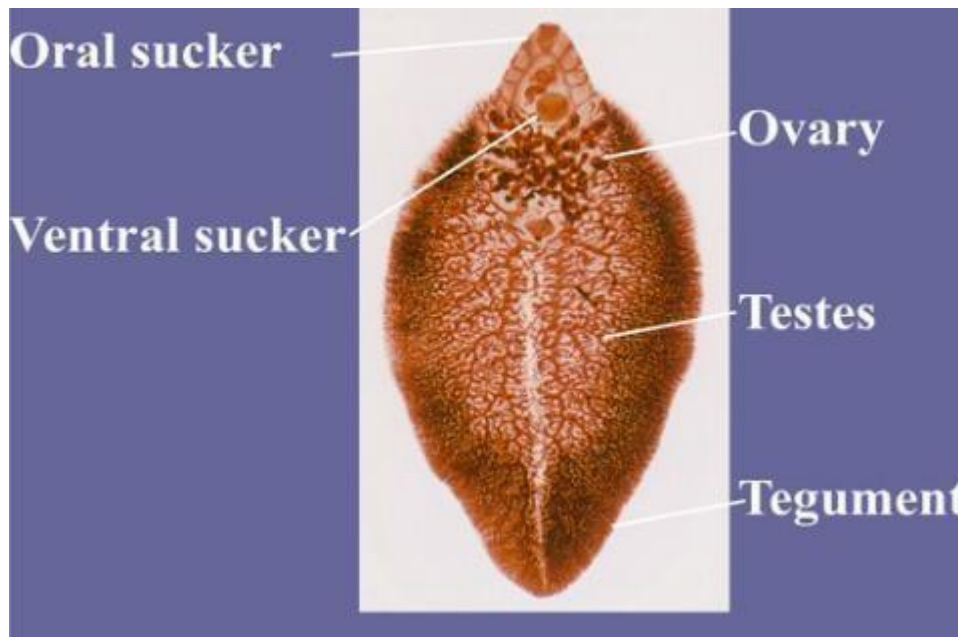


TAPEWORM

Adult tapeworms are flattened, elongated, and consist of segments called proglottids. Tapeworms vary in length from 2 to 3 mm to 10 m, and may have three to several thousand segments. Anatomically, cestodes are divided into a scolex, or head, which bears the organs of attachment, a neck that is the region of segment proliferation, and a chain of proglottids called the strobila.

FLUKES(Trematodes)

Flukes or trematodes are leaf shaped and vary in length from a few millimeters to 7 to 8 cm. Excluding blood flukes, trematodes are hermaphroditic, having both male and female reproductive organs. Both self-fertilization and cross-fertilization occur. Blood flukes (schistosomes) are the only bisexual flukes that infect humans. The tegument is morphologically and physiologically complex.



FLUKE (TREMATODES)

SIGNS AND SYMPTOMS

Helminthiasis is observable generally in case of mild infections. Long-term infections can be detected with symptoms like;

- Abdominal pain
- Loss of appetite
- Weight loss
- Cough
- Visible worms in the stool (in some cases)

Apart from these Helminthiasis symptoms, other signs of helminthiasis include rectal prolapse. In cases where worms in the stool are not visible to the naked eye, stool samples tests confirm the presence of eggs.

ROUNDWORMS

Human roundworm infection or *Ascaris* mainly affects the small intestine and is extremely common among children who live in unsanitary environments. And

the main symptoms are

- Nausea
- Vomiting
- Abdominal pain
- Diarrhoea
- Loss of appetite
- Visible worms in the stools
- Weight loss

Severe helminthiasis symptoms such as unbearable abdominal pain, fatigue, and fever, indicate that the condition is very severe.

HOOKWORMS

Hookworms are helminths that affect your small intestine, lungs, and skin. According to the CDC, around 576-740 million people globally have hookworm infections. again it is most common among children. here are common helminthiasis symptoms caused by hookworms.

- Abdominal pain
- Diarrhoea
- Excessive crying in small children
- Fever
- Nausea and vomiting
- Loss of appetite
- Itchy rashes
- Blood in stools

WHIPWORMS

Unlike roundworms and hookworms infection the whipworms affects the large intestine. Around 600-800 million people in the world have whipworm infection. It is a very common helminth found in unsanitary tropical conditions. Here are the main symptoms of a whipworm intestinal helminthiasis infection.

- Abdominal pain
- Bloody diarrhea
- Nausea
- Vomiting
- Painful and frequent defecation

COMPLICATIONS

A lot of complications can occur in helminth infection, which may include.

- Anemia
- Malnutrition
- Growth retardation
- Developmental retardation
- Intestinal obstruction

MANAGEMENT

NONPHARMACOLOGICAL MANAGEMENT

Helminthiasis occurs in region with poor sanitation and hygiene. Therefore, practicing simple hygiene practices and keeping the environment clean will make a huge difference in keeping away parasitic worm infection.

- Avoid eating raw, uncooked vegetables. Always wash vegetables thoroughly even before cooking.
- Avoid eating raw and unprocessed meat. Make sure the purchased meat from reliable sources that guarantee cleanliness and follow a strictly safety protocol
- Disinfect utensils and surfaces that have come in contact with raw meat.
- Avoid walking barefoot on the soil, especially in unhygienic places.
- Get rid of feces and other animal waste in your surroundings.

TREATMENT

CLASSES OF ANTIHELMINTIC DRUGS

Anthelmintics and nematocides are separated into classes on the basis of similar chemical structure and mode of action. There are only a few main classes and each is briefly discussed in turn below.

The easiest way to organize these drugs is to consider a reasonable organization of the worms. The helminths (worms) are classified into three groups. Cestodes (flatworms), nematodes (roundworms), and trematodes (flukes).

HELMINTH	DRUG OF CHOICE
Cestodes (flatworms and tapeworm)	Praziquantel
Trematodes (flukes, schistosomiasis)	Praziquantel
Nematodes	Albendazole Mendazole
Roundworms	Diethylcarbamazine
Filariasis	Ivermectin

LITERATURE SURVEY



MORPHOLOGY OF CHINESE PETAI (LEUCAENA LEUCOCEPHALA)

SCIENTIFIC CLASSIFICATION

KINGDOM: Plantae
 CLADE: Tracheophytes
 CLADE: Angiosperms

CLADE: Eudicots
 CLADE: Rosids
 ORDER: Fabales
 FAMILY: Fabaceae

SUBFAMILY

Caesalpinioideae
 ADE : Mimosoid clade
 GENUS : *Leucaena*
 SPECIES : *L. leucocephala*

SYNONYMS

- ❖ *Acacia frondosa* Willd.
- ❖ *Acacia glauca* (L.) Willd.
- ❖ *Acacia leucocephala* (Lam.) Link
- ❖ *Mimosaleucocephala* Lam.
- ❖ *Mimosaleucophala* Lam.

VERNACULAR NAMES

ENGLISH: Wild tamarind, Lead tree
 HINDI: Subabul, Kadam
 ARABIC: *Leucaena*
 FRENCH: Fauxmimosa, Leucene
 HINDI: Kadam, Toira, Subabul
 CHINESE: Yinhehuan
 SPANISH: Guaje, Peruleucaena, Acacia
 bellarosa MEXICO : liliak

SYNONYMS

Acacia leucocephala (Lam.) Link
 Leucaena glauca (L.) Benth. (misapplied)
 Mimosa leucocephala Lam.

SIMILAR SPECIES

There are two sub-species of *Leucaena leucocephala*.
L. leucocephala subsp. *leucocephala* and *L. leucocephala* subsp. *glabrata*.
 These two sub-species can be distinguished by the following differences.

- *L. leucocephala* subsp. *leucocephala* is a relatively small and much-branched tree with younger stems that are densely covered with fine greyish-coloured hairs (they are puberulous).
- *L. leucocephala* subsp. *glabrata* is a relatively large and sparsely-branched tree with younger stems that are hairless (glabrous).

Other *Leucaena* species have been introduced to East Africa such as *L. diversifolia*, *L. pallida* and *L. trichandra*. They are all probably invasive.

DESCRIPTION

Leucaena leucocephala is a shrub or small tree usually growing 2-10 m tall, but occasionally reaching 15 m or more in height. The leaves (up to 35 cm long) are twice-compound (bipinnate) and have 3-10 pairs of branchlets (pinnae). They are alternately arranged along the stems and borne on stalks (petioles) 2-5 cm long. A small raised structure (gland) is usually present on the leaf stalk (petiole), or just below where the lowest pair of branchlets (pinnae) meet. Pinnae are 2-10 cm long and each bears 5-22 pairs of leaflets (pinnules). These leaflets (7-21 mm long and 1.5-5 mm wide) are elongated (narrowly-oblong to lanceolate) in shape with pointed tips (acute apices), and are either hairless (glabrous) or have hairy (ciliate) margins.

DISTRIBUTION

There is no doubt that *L. leucocephala* originates from Mexico. However, its true natural distribution is extremely difficult to ascertain in detail because it is cultivated throughout Mexico and Central America and no unambiguously natural populations have so far been located. Thus, earlier references to *L. leucocephala* being native to Guatemala and Belize are not included here.

USES

Leucaena leucocephala is widely cultivated, mostly in farming situations, for forage (leaves and shoots), firewood, poles, medicine (roots), shade, soil conservation and improvement, tannin, dye. It is also planted as a windbreak, a garden ornamental and as an urban shade tree. In Uganda, it was introduced in tea plantations and as a host for the vanilla orchid (*Vanilla planifolia*) and later planted among other crops as a nitrogen fixer.

SCOPE OF WORK

The aim of present study is to evaluate the anthelmintic activity of ethanolic extract of leaves of *leucaena leucocephala* leaves. The work was undertaken in the department of pharmacognosy, DR. KVSP INSTITUTE OF PHARMACY, KURNOOL.

The main objective of present study was

- Collection and authentication of *leucaena leucocephala* leaves.
- Successive ethanol extraction of the drug.
- Phytochemical screening of the extracts.
- Evaluation of anthelmintic activity of *leucaena leucocephala* leaves.

MATERIAL AND METHODS**Collection and authentication of plant material**

The plant material of *Leucaena leucocephala* leaves were collected from the surrounding regions of Dupadu area of kurnool district. The plant material was cleaned properly, at first it was washed with tap water and then with pure or distilled water to remove all the dust particles or impurities and then kept in shade to dry properly and crushed to coarse powder. This plant species was identified and authenticated as *Leucaena leucocephala* by St. Joseph's Degree College.

PREPARATION OF PLANT EXTRACTS

The plant material was properly cleaned, at first washed with tap water and then with pure or distilled water to remove all impurities or dust particles and then kept in shade to dry properly. Powdered material was then extracted in "Maceration" process using ethanol respectively. About 50 gms of powdered leaves of *leucaena leucocephala* was extracted with 150 ml of ethanol (40 °C) using maceration process for 72 hours. Then the extracts were concentrated to semi-solid masses and stored in an air sealed container in a refrigerator for further uses.



Ethanolic extract of *leucaena leucocephala*

ANIMALS

Adult Indian earthworm (*Pheretima posthuma*) were obtained from vermipost, kurnool and washed with normal saline to remove all adhering faecal or unwanted matter which were further used for the study of anthelmintic activity. The earthworms *P. posthuma* were

about 5-7 cm long and 0.2-0.4 cm wide. They were used for all experimental procedures because they have anatomical and

physiological similarities with intestinal roundworm parasite present inside human beings.



**PHERETIMA POSTHUMA
(EARTHWORMS)**

DRUGS AND CHEMICALS

DRUG

TINIDAZOLE And all the other solvents and chemicals used during experimental protocol were analytical grade.



TINIDAZOLE



MACERATION PROCESS

Phytochemical analysis of the prepared extracts

The preliminary phytochemical analysis of the leaves of *L. leucocephala* plant extract mainly done for the evaluation of the various phytochemical constituents such as anthraquinones, alkaloids, flavonoids, proteins, sugars, sterols, tannins, Saponins and terpenoids were present in plant extracts prepared in solvents Ethanol of *L. leucocephala*.

TEST FOR ALKALOIDS

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtered were tested carefully with alkaloids reagents.

Mayer's Test

Took 2ml plant extract and 2ml concentrated HCL were added. Mayer's reagent was further added in a little amount. Green colour or white precipitate obtained which shows that the alkaloid groups are present.

Test for Anthraquinone

1g of the plant extract was at first boiled with 20 ml of H₂SO₄ and filtered during heated state. The filtrate material was shaken-up with 10 ml chloroform. The

layer of chloroform was pipetted into another test tube and 2 ml dilute ammonia (NH₄) was added. Now the prepared solution was kept and observed for changes in colour.

Test for Flavonoids (Ferric chloride test)

Took approx. 1g of the plant extract and boiled in 10 ml pure or distilled water then filtered. 4ml of filtrate was taken and few drops of 10% ferric chloride solution was added. Violet or Green-blue colour obtained which indicates the existence of a phenolic hydroxyl group.



FERRIC CHLORIDE TEST

Test for proteins (Xanthoproteic test)

Little amount of the plant extract was dissolved in 4 ml distilled water, 1 ml concentrate nitric acid (HNO₃) further added in the solution. Yellow colour obtained which shows the presence of "proteins".



PROTEIN TEST

Test for Sugars (Fehling's test for free reducing sugar)

Took about 1g of plant extract and dissolved in pure or distilled water, then filtered. Now the filtrate material was heated with 10 ml of Fehling's solution A and B separately. Red coloured precipitate of cuprous oxide (Cu₂O) formed which shows that the reducing sugars

are present.

Test for sterols (Salkow's reaction)

Few mg of the plant extract was dissolved in 1 ml chloroform, then 1 ml of concentrated sulphuric acid (H₂SO₄) was added. The test tube was vigorously shaken-up for up to 3-4 minutes. Red colour appeared in the chloroform layer, indicating the presence of "sterols".

Test for tannins (Ferric chloride reagent test)

The plant extract was taken on an individual basis in pure or distilled water, warm and filtered. Took a little volume of the filtrate and added some drops of 5% w/v solution of ferric chloride, prepared in 90% alcohol. A deep green or blue colour appeared, indicating that the tannins are present.

Test for Saponins

2 g of the plant extract taken and boiled with 10 ml of pure or distilled water, then filtered. Took the filtrate, added about 6ml of pure or distilled water and vigorously shaken for up to 5 minutes. Foaming which comes on warming indicates the existence of saponins.

Test for terpenoids(Salkowskitest)

Took 1 g of extract added 4 ml of chloroform, a further addition of 6 ml of concentrated Sulphuric acid to form a layer. A reddish brown colour of the interface appeared which indicates the presence of terpenoids.

ANTIHELMINTHICACTIVITY

Ethanol extract was explored for antihelminthic activity according to the method described by amit sharma, adult indian earthworms (*Pheretima posthuma*), due to their physiological and anatomical resemblance with human beings parasites (intestinal roundworm) are used in present study. The worms were divided into group containing six earthworms in each. ethanolic extract was diluted to concentration of 10, 15, 20mg/ml with DMSO. Tinidazole (standard drug) was dissolved in

DMSO to make 10, 15, 20mg/ml. All the solutions were prepared freshly prior to the experiment. earthworms were washed with normal saline and transfer to petridishes containing of different concentration extracts and standard (10mg/ml).

Observations were made for the time taken for paralysis (when no movement of worms could be observed except when the worms were shaken vigorously) and death (worms neither move on shaking vigorously).

RESULTS**COLLECTION AND IDENTIFICATION OF DRUG**

The leaves of *Leucaena leucocephala* to family (Fabaceae) were selected for the study. The plant material was collected from local areas of Kurnool and authenticated by St. Joseph Degree College.



ETHANOLIC EXTRACT OF LEUCAENA LEUCOCEPHALA

Table: Colour, nature and percentage yield of the extract.

Name of the plant	Weight of powder of leaves	Extract	Colour	Consistency	Yield
					Wt %
<i>Leucaena leucocephala</i>	50gms	Ethanolic Extract	Greenish Black	Semi solid And Non-Sticky	6.020

PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACT OF LEUCAENA LEUCOCEPHALA

PHYTOCHEMICAL TESTS	TEST USED	ETHANOL
Alkaloids	Mayer's test	+
Anthraquinones		+++
Flavanoids	Ferric chloride test	++
Proteins	Xanthoproteic test	+++
Tannins	Ferric chloride reagent test	+++
Terpenoids	Salkowsky's test	+++
Saponins	Foam test	-
Sterols	Salkowsky's test	++
Sugars	Fehling's solution test	+++

NOTE: Slightly present

(+) Moderately present (++) Significantly present (++++)

EXPERIMENTAL DESIGNS

Ethanol extracts were suspended in 0.5% concentrated solution of DMSO (Dimethylsulphoxide)

which is prepared in pure ethanol. All the solutions and extracts were freshly prepared before the starting of the experiment. 16 groups each were containing 6

earthworms, released into 10 ml of desired formulation as-

Group I were the control worms placed in vehicle 0.5% DMSO in ethanol.

Groups II-IV received ethanolic extracts of *L. leucocephala* at 10 mg/ml, 15 mg/ml and 20 mg/ml concentrations respectively.

Group V-VII treated with tinidazole at 10, 15 and 20 mg/ml concentrations respectively. The final volume was set to 10 ml in each of the petridish. Observations were prepared on the basis of the time occupied to paralyse and cause death of each worm individually during the test period. The occurrence of Paralysis was reported when the worms did not survive even in the normal saline. Death was determined when the worms lost motility indicated by fading their body colour.

IN-VITRO ANTIHELMINTIC ACTIVITY

Effect of different extracts at time of paralysis

Ethanolic extract exhibited better anthelmintic activity when compared with the standard drug Tinidazole at the same concentrations and conditions.

Effect of different extracts at time of death

Ethanolic extract exhibits better anthelmintic activity when compared with standard synthetic drug at the same concentration and condition. Ethanolic extract occupied minimum time to cause death of the worms. If 10 mg/ml dose of tinidazole drug is compared with ethanolic extract then it can be determined that plant extract contains better effectiveness as compared to synthetic drug for anthelmintic activity.

Standard Drug Concentrations Ethanolic Extract Concentrations



10mg/ml



10mg/ml



15mg/ml



15mg/ml



20mg/ml

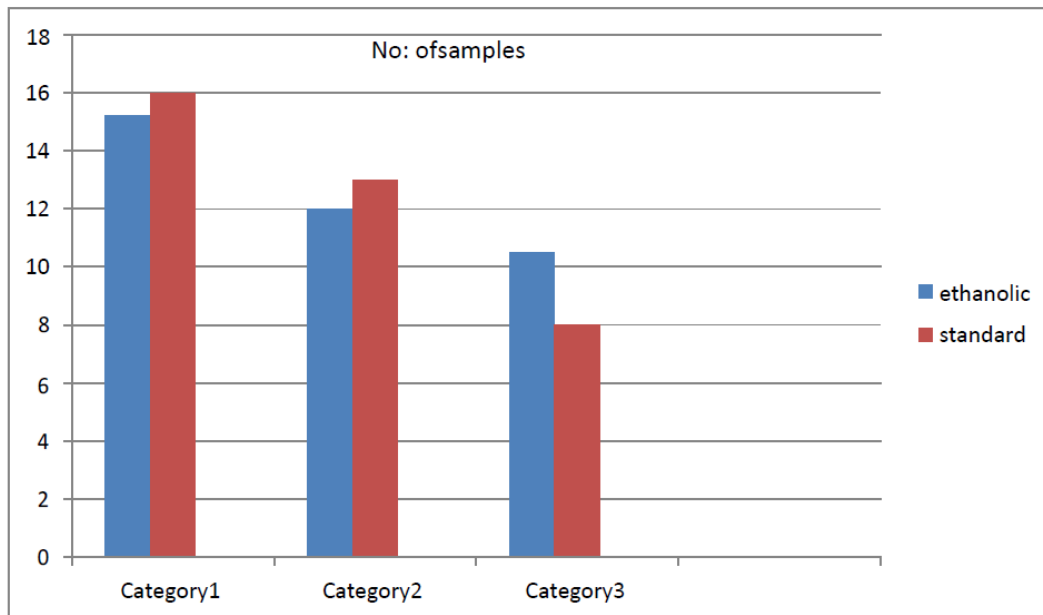


20mg/ml

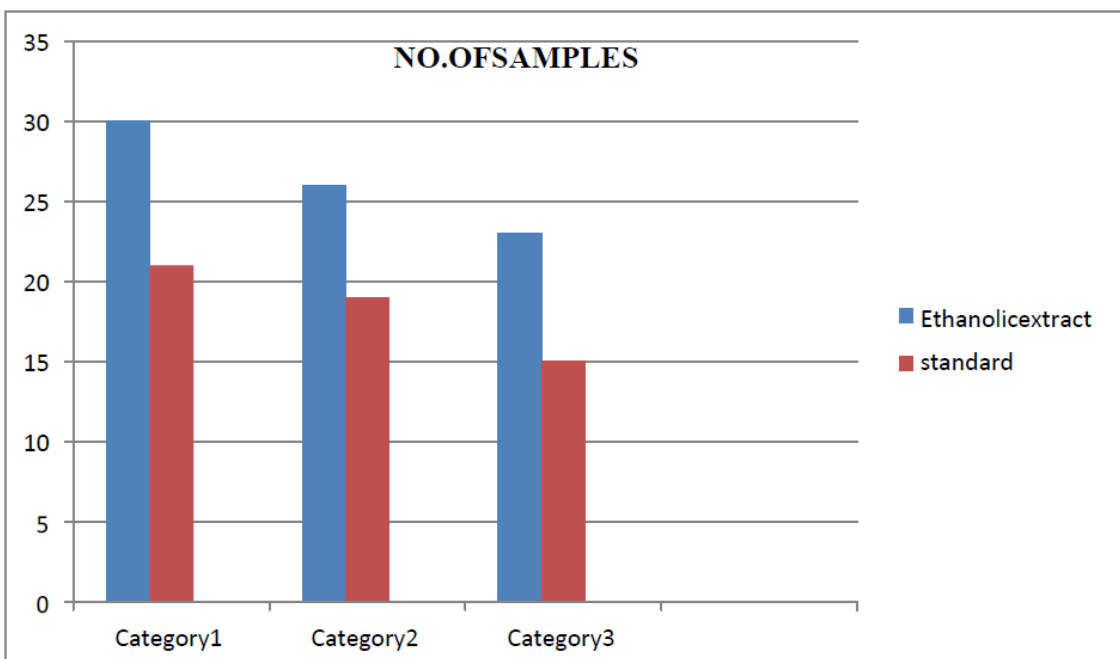
Anthelmintic activity of ethanolic extract of *flucaena leucocephala*.

TREATMENT	DOSE(Mg/ml)	Time taken for paralysis of earthworm	Time taken for death of earthworms
Vehicle	-	-	-
Ethanolic extract	10mg/ml	15.2 min	30 min
	15mg/ml	12 min	26 min
	20mg/ml	10.5 min	23 min
Tinidazole	10mg/ml	16 min	21 min
	15mg/ml	13 min	19 min
	20mg/ml	8 min	15 min

PARALYSIS TIME OF 10mg/ml, 15mg/ml, 20mg/ml of extracts and standard



DEATH TIME OF 10mg/ml, 15mg/ml, 20 mg/ml of extract and standard



DISCUSSION

The data revealed that the various extracts showed paralysis and time of death at a concentration of 10mg/ml, 15mg/ml, 20mg/ml in concentration dependent manner. The test concentration of all the extracts showed marked degree of anthelmintic activity with maximum activity of ethanolic extract. The anthelmintic effect of extract is compared to the effect produced by the standard drug tinidazole.

Marvellous researches have been done during the previous decade and large numbers of synthetic precursors have been derived to cover the damage caused by parasites. But unfortunately no effective medicine has been developed till now. Some severe side effects of drug and development of resistance increases the severity of infection to the next level. These factors considered the way for herbal remedies as alternative anthelmintic. The result of this study has shown promising anthelmintic activity suggesting the possible use of *Leucaena leucocephala* extract in control of intestinal nematode.

CONCLUSION

This study has demonstrated that the various leaf extracts of *Leucaena leucocephala* possess significant in vitro anti-worm activity at the tested concentrations. The ethanolic extract shows maximum activity at all the tested concentration. Thus, the wormicidal activities of the plant extract against earthworms suggest that it can be effective against parasitic helminths of humans and animals. However, further studies are needed to isolate, characterize and evaluate the actual bioactive components and their mechanism of action. Also, studies on the toxicity, evaluation of the effect in-vivo and the establishment of adequate doses for human and animals are recommended.

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