



Original Research Article

.Assessment of ethanol leaf extract from urtica dioica for in vitro Anti-Helminthic properties

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ABSTRACT

Helminthic infections continue to be a significant public health concern worldwide, particularly in regions with poor sanitation and limited access to healthcare. With the increasing prevalence of drug resistance among helminths, there is a pressing need for alternative treatments derived from natural sources. *Urtica dioica*, commonly known as stinging nettle, has been traditionally used for various medicinal purposes, including its purported anthelmintic properties. This study aimed to evaluate the in vitro anti-helminthic activity of ethanol leaf extract of *Urtica dioica* against helminth parasites. Fresh leaves of *Urtica dioica* were collected, dried, and powdered. The ethanol leaf extract was prepared using standard extraction methods. Various concentrations of the extract were tested against helminth parasites using established in vitro assays. The efficacy of the extract was determined by assessing parameters such as paralysis and mortality of the parasites. Preliminary findings indicate that the ethanol leaf extract of *Urtica dioica* possesses significant anti-helminthic activity against the tested parasites. The extract exhibited dose-dependent paralysis and mortality of the helminths, with higher concentrations demonstrating greater efficacy. These results suggest the potential of *Urtica dioica* as a natural source of anthelmintic agents. Further studies are warranted to elucidate the specific bioactive compounds responsible for the observed anti-helminthic activity and to evaluate the safety and efficacy of *Urtica dioica* extract in vivo. Additionally, investigations into the underlying mechanisms of action and potential synergistic effects with conventional anthelmintic drugs may provide valuable insights for the development of novel therapeutic interventions against helminthic infections. The study results when compared to standard drug of fenbendazole, ethanolic extract of *Urtica dioica* possess a good significant anti-helminthic activity compared to standard drug of fenbendazole.

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1. Introduction

Helminthiasis is a macro parasitic disease of human and animals in which a part of body is infested with the parasitic worms such as pinworm, roundworm or tapeworm. Intestinal parasitic helminths such as roundworms (*Ascaris lumbricoides*), hookworms (*Ancylostoma doudenale* and *Necator americanus*) and whipworm (*Trichuris trichiura*)

are common in the developing world. The prevalence of intestinal worm infestation in India varies from 5% to 76%, which is similar to that in other developing countries. These parasitic infestations are acquired by ingestion, inhalation or penetration of the skin by the infective forms. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs. Infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation. Other people can then be infected by ingesting eggs or

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larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Infestation can cause morbidity and sometimes death, by compromising nutritional status, affecting cognitive processes, including tissue reaction, such as granuloma, and provoking intestinal obstruction or rectal prolapse.¹

Helminth infections are among the most widespread infections in humans, distressing huge population of the world. About 3.5 billion people in the world are affected and 450 million are ill as a result of parasitic infection. The diseases caused by helminth infection are often called tropical diseases. They are in fact diseases of under development since the common feature of the societies in which helminth infection are highly prevalent is low socioeconomic status. In tropical regions, where prevalence is greatest, simultaneous infection with more than one type of helminth is common.¹ The majority of the infections due to helminths are generally restricted to tropical regions and cause enormous hazards to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia.

Some examples of the effects of the helminth infection in man:²

1. Mechanical obstruction- large ascaris burden can cause mechanical or spastic.
2. Obstruction of the intestine and produce serious or fatal illness.
3. Hypersensitivity- the antigens shed by ascaris larvae as they migrate through the lungs can induce antibody formation and cause severe immediate hypersensitivity or asthma.
4. Anaemias – hookworm infection leads to morbidity by blood loss, iron deficiency anaemia and hypoproteinaemia which can be serious in individuals already malnourished.³
5. Malnutrition – helminth infection can have adverse effect on the nutritional status especially of individuals who are already suffering from starvation. The mechanism by which helminth infection can adversely affect nutritional status are complex, but may include: loss of appetite; decreased absorption of essential nutrients such as iron and vitamins; host-parasite competition for food materials; and diarrhoea.
6. Upper gastrointestinal haemorrhage due to portal hypertension caused by *S. mansoni* is a major cause of mortality among adult males in Egypt.
7. Carcinoma of the bladder associated with chronic Schistosoma haematobium infection is a common malignancy in Egyptian men.

1.1. Roundworms

Roundworms are a member of the nemathelminth's phylum or group of animals. The hookworm, pinworm and

trichinella are part of this group. They are more advanced than flatworms but less advanced than earthworms. They have thin round bodies, with none of the pieces or segments that earthworms have in their bodies.

Roundworms live in salt water, fresh water and the soil. Many of them are harmful to man as they are parasites.

1.2. Scientific classification

Kingdom- Animalia

Clade- Nematoda

Phylum- Nematoda

1.3. Anatomy and physiology of roundworms⁴

Muscular Skeletal - A roundworm has no skeleton.

Digestion - A roundworm has a definite digestive system that runs the length of their bodies. It has a mouth, pharynx, intestine and anus. Many are parasites and live off other animals and plants.

Nervous - A roundworm has two nerve cords that transmit impulses in the roundworm. Circulation - A roundworm has no heart or formal blood vessels.

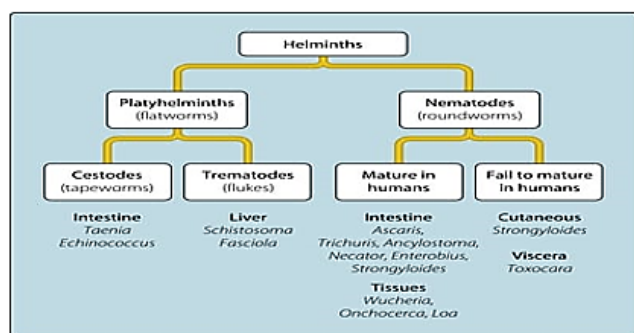
Respiration - A roundworm has no formal respiratory system.

Reproduction - A roundworm reproduces sexually. The female has an ovary, holds eggs in an oviduct and then passes them to the uterus, where they are fertilized. When it is time to reproduce, the sperm cells pass through the spicule. Over 200,000 eggs can be deposited at once in the soil once they are refertilized.

Excretion - A roundworm has an anus at its rear end and a series of excretory tubes that end in an excretory pore.

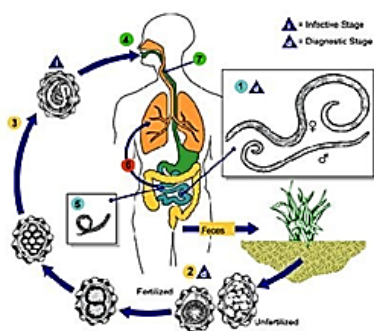
Symmetry - A roundworm has bilateral symmetry.

Appearance - A roundworm is thin, round, smooth and can be up to four feet in length.



Appearance - A roundworm is thin, round, smooth and can be up to four feet in length.

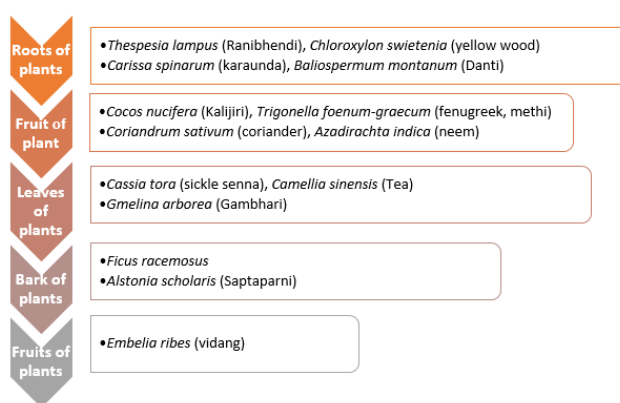
Life cycle



- Step 1: Adult worms live in the lumen of the small intestine.
- Step 2 and 3: Fertile eggs embryonate and become infective after 18 days to several weeks
- Step 4: The eggs of the worm are found in soil contaminated by human feces or in uncooked food contaminated by soil containing eggs of the worm. Humans are infected
- Step 5: The eggs hatch into larvae within the person's intestine.
- Step 6: The larvae penetrate the intestine wall and reach the lungs through the blood stream.
- Step 7: The larvae mature further in the lungs (10 to 14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed. Upon reaching the small intestine, they develop into adult worms.⁵

1.4. Herbal medicinal plants used as anthelmintic

Many herbs in different parts are used as natural anthelmintics like



1.5. Pharmacotherapy⁶

Table 1: Broad spectrumanthelminti

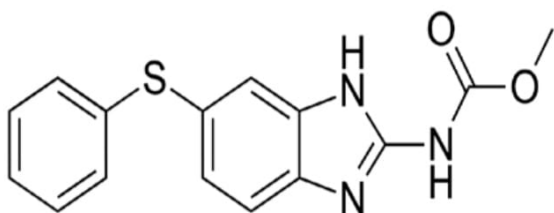
Drugs effective in roundworms, threadworms, hookworms, tapeworms and flukes	Drugs effective in roundworms, threadworms and hookworms
Eg: Mebendazole	Eg: Thiabendazole, Albendazole, Pyrantel pamoate

Table 2: Narrow Spectrumanthelmintics: Drugs effective against

Round worm & threadworm	Hook worm	Filariasis	Tapeworm	Flukes
Eg:Piperazine, Tetramisole or Levamisole, Ivermectin, Santonin andHexylresorcinol	Eg: Bephenium, Thymol, Chenopodium	Eg: Diethylcarbamazine	Eg: Niclosamide, Meoacrine and Chloroquine	Eg: Praziquantel, Bithionol, Metrifonate and Oxamniquine

Fenbendazole is a member of the class of benzimidazoles that is 1H-benzimidazole which is substituted at positions 2 and 5 by (methoxycarbonyl)amino and phenylsulfamide groups, respectively. A broad-spectrum anthelmintic, it is used, particularly in veterinary medicine, for the treatment of Nematoda infections carbamate

FENBENDAZOLE



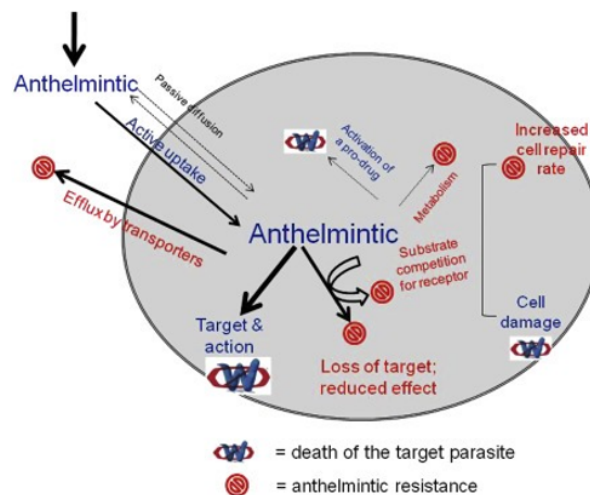
2. Medicinal Uses

- Anthelmintic:** Fenbendazole is primarily used as an anthelmintic medication to treat parasitic worm infections in animals. It is effective against various types of worms including roundworms, hookworms, whipworms, and certain types of tapeworms.
- Veterinary Medicine:** Fenbendazole is commonly used in veterinary medicine to treat parasitic infections in dogs, cats, horses, livestock, and other animals.
- Anticancer Properties:** Preliminary studies and anecdotal reports suggest fenbendazole may have potential anticancer properties, but further research is needed to fully understand its safety and efficacy for this purpose in humans.
- Fenbendazole is a broad-spectrum anthelmintic (anti-parasitic) drug commonly used in veterinary medicine to treat various parasitic infections in animals. It belongs to the benzimidazole class of compounds. Here is a drug profile of fenbendazole:
- Mechanism of Action:** Fenbendazole acts by disrupting the microtubule structure in the cells of parasites, thereby inhibiting their ability to divide and reproduce. This leads to the death of the parasites.
- Administration:** Fenbendazole is typically administered orally as a tablet, paste, or liquid suspension. The dosage and duration of treatment vary depending on the type of infection and the species of the animal being treated.
- Side Effects:** Although fenbendazole is generally considered safe when used as directed, some animals may experience side effects such as:
 - Vomiting
 - Diarrhea
 - Loss of appetite
 - Lethargy
 - In rare cases, allergic reactions or neurotoxicity may occur

3. MOA of Fenbendazole

3.1. Mechanism of action

Fenbendazole acts by disrupting the microtubule structure in the cells of parasites, thereby inhibiting their ability to divide and reproduce. This leads to the death of the parasites.



3.2. Mechanism of action of anthelmintic Drugs (Fenbendazole)

Overall, *Urtica dioica* exhibits a range of pharmacological activities, making it a subject of interest for further research and potential therapeutic applications. However, it's essential to consult with a healthcare professional before using stinging nettle for medicinal purposes, especially if you have any underlying health conditions or are taking medications.³

Anti-inflammatory: Stinging nettle contains compounds like flavonoids and phenolic acids that have been found to possess anti-inflammatory properties. This makes it useful in traditional medicine for conditions such as arthritis and allergic rhinitis.

Diuretic: The plant has been traditionally used as a diuretic, promoting urine production and helping to flush out toxins from the body. This diuretic activity is beneficial for conditions such as urinary tract infections and edema.

Antioxidant: Stinging nettle contains antioxidants like flavonoids, which help neutralize free radicals in the body, reducing oxidative stress and inflammation. This antioxidant activity contributes to its overall health-promoting effects.

Antimicrobial: Some studies have shown that extracts of stinging nettle possess antimicrobial properties, inhibiting the growth of certain bacteria and fungi. This activity may contribute to its traditional use in treating infections.⁷

Hypoglycemic: Research suggests that stinging nettle may have hypoglycemic effects, helping to lower blood sugar levels. This potential activity could be beneficial for managing diabetes and related complications.

Hypotensive: There is evidence to suggest that stinging nettle may have hypotensive effects, meaning it can help lower blood pressure. This activity may be attributed to its vasodilatory properties.

Plant Study/ Taxonomy of *Urtica dioica*:



Kingdom	Plantae
Phylum	Angiosperms
Class	Eudicots
Order	Rosales
Family	Urticaceae
Genus	<i>Urtica</i>
Species	<i>Urtica dioica</i>
Synonym	<i>Urticamajor/urens/ galeopsifolia, procera, hirsute, gracilis</i>
Common names	Stinging nettle, Common nettle, Nettle, Stinger, Nettle leaf, European nettle

3.3. Description

Urtica dioica, commonly known as stinging nettle, is a perennial herbaceous plant characterized by its serrated, heart-shaped leaves and its ability to deliver a painful sting upon contact with its tiny, hair-like structures containing irritants. The plant typically grows to a height of 1 to 2 meters and thrives in disturbed areas, woodlands, meadows, and along streams.

Stinging nettle has a long history of use in traditional medicine and cuisine. Its leaves are edible when cooked

or dried and are rich in vitamins, minerals, and protein. Medicinally, it has been used to treat a variety of ailments such as arthritis, allergies, urinary tract infections, and skin conditions due to its anti-inflammatory and diuretic properties.

Overall, *Urtica dioica* is a versatile plant with both beneficial and defensive attributes, making it an important species in various ecological and cultural contexts

Urtica dioica has a rich ethnobotanical history and various traditional uses:

Medicinal Uses:⁸ Stinging nettle has been used medicinally for centuries. It is believed to have diuretic, anti-inflammatory, and antioxidant properties. It has been used to treat conditions such as arthritis, allergies, urinary tract infections, and skin irritations.

Food Source: Despite its stinging hairs, stinging nettle is edible when cooked or dried. Its young leaves can be harvested and used in soups, teas, or as a spinach substitute. It is rich in vitamins, minerals, and protein.

Fiber Source: Historically, the fibers from stinging nettle stems have been used to make textiles. The plant's fibers are strong and have been used to make cloth, rope, and paper.

Cultural and Ritual Uses:⁹ In some cultures, stinging nettle has been used in rituals or as a protective charm. Its stinging properties were believed to ward off evil spirits or provide protection against negative energies.

Wildlife Habitat: Stinging nettle provides habitat and food for various insects, including butterfly larvae, which feed on its leaves.

3.4. Chemical constituents

Flavonoids	Phenolic compounds	Amino acids	Minerals	Vitamins	Lignans	Triterpenes	Acids
Quercetin, kaempferol, rutin	Chlorogenic acid, caffeic acid, ferulic acid	Histidine, lysine, phenylalanine, serine	Calcium, magnesium, iron, potassium	Vitamin A, c & K	Secoisolariciresinol	β -sitosterol, stigmasterol	Linoleic acid, linolenic acid, palmitic acid, stearic acid
antioxidant properties	anti-inflammatory properties	metabolic processes	physiological functions in the human body	immune function	hormone-balancing effects	anti-cancer properties	lipid metabolism and cellular function

4. Aim & Objectives

The aim of the present study is to evaluate invitro anthelmintic activity of ethanolic extract of *Urtica*.

Following are the objectives of the study

1. To Identify plant *Urtica dioica* and authentication of plant
2. To collect all equipment's and laboratory chemicals required for study Collect leaves of *Urtica dioica* and prepare ethanolic leaf extract
3. Drying of ethanolic leaf extract by rotary evaporator

4. To carry out phytochemical screening for ethanolic plant extract
5. To collect Indian earthworms
6. To carry out in-vitro study on earthworms

5. Experimental Design/ Plan of Work

5.1. Plant collection

1. Plants are collected from the nearby institution surroundings
2. Plants claimed with anthelmintic activity as selected for the proposed study and suitable plant part leaf ethanolic extract is prepared.

5.2. Animals

The anthelmintic activity was performed on adult Indian earth worm “*Pheretima posthuma*” it has anatomical and physiological resemblance with the intestinal round worm of human beings’ Indian earthworms are collected from vermicompost area of Nallajerla, east Godavari district. All the Earth worms are handled with gloves and placed in sterilized Petri dishes.

5.3. Drugs and chemicals

Fenbendazole tablets was procured in surrounding pharmacy area of Nallajerla. All other laboratory chemicals required ethanol 90% for phytochemical screening was procured National Scientific products (NSP).

5.4. Instruments

Weighing balance, gloves, whattsman filter paper, and mortar and pestle, petridishes, droppers, heating mantle, filtration equipment.

6. Methodology

6.1. Preparation of plant extract

The crude ethanolic extract was prepared by the simple decoction, method using 20 g of triturated dried leaves for 300ml of ethanol After 30- mints of decoction the whole content was blended in a domestic blender filtered by whattsman filter papers extract obtained was evaporated in rotary evaporator.¹⁰

6.2. Preliminary phytochemical screening

Ethanolic extract obtained by above procedure is subjected to phytochemical screening of qualitative analysis of phytoconstituents.



7. Experimental Design²

Experimental was carried out on 42 adult Indian earthworms randomly divided into nine groups with distilled water, extract(Plant)&Fenbendazole (Brand name Pana cur).

1. Group I receive normal saline (Nacl water in the study acts as a control
2. Group II receiving standard drug fenbendazole is sub divided into groups receiving 10%, 20%, 30% of standard drug.
3. Group III receiving test dose of plant extract are further separated into group receiving 10% 20% 30% of ethanolic plant extract.

All the animals treated with control, standard and plant extract was observed for time taken for paralysis when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water All the results were shows in Table 1 and expressed as a mean SEM of six worms in each group.

8. Results

The percentage yield was obtained using this formula:

1. $W2W1/W0DOCXUPLOAD:INLINE:088b05f4cc324baf9ff241d$
c.p, c.p et al)
2. Where W2 is the weight of the extract and the container.
3. W1 the weight of the alone.
4. W0 the weight of the initial dried sample.

Table 3: Test for Alkaloids

S. No	Test	Observation	Inference
1	Wagner's test -1.27 gm of iodine +2 gm of potassium iodide made up to 200 ml.	Reddish brown precipitate	Presence of alkaloid
2	Mayer's Test - 1.36 gm of mercuric chloride +5 gm of potassium iodide in 60 ml of distilled water and made up to 200 ml.	White to buff precipitate	Presence of alkaloid
3	Dragendroff's Test - 14 gm of sodium iodide +5.2 gm of bismuth carbonate in 50 ml glacial acetic acid. 40 ml filtrate+160 ml of acetate +1ml of water. 10 ml of stock +20 ml of acetic acid made up to 100 ml with water.	Orange – brown colour precipitate	Presence of alkaloid
4	Hager's Test -Saturated aqueous picric acid	Crystalline precipitate with many precipitates	Presence of alkaloids

Table 4: Test for carbohydrates

SNO	Test	Observation	Inference
1	Benedict's Test-5 ml of benedict's reagent (benedict's A+ benedict's B) +3ml of test solution on a water bath	Brick red precipitate at bottom of the test tube	Presence of carbohydrates
2	Fehling's Test- 2 ml of Fehling A+2 ml of Fehling B + 2ml of extract were boiled	Yellow or brick red precipitate at the bottom of test tube	Presence of carbohydrates
3	Molisch's Test- Aqueous or alcoholic solution of the extract +10% alcoholic solution of α -naphthol+ concentrated sulphuric acid	A violet ring at the junction of two liquids	Presence of carbohydrates

Table 5: Test for cardiac glycosides

SNO	Test	Obsevation	Inference
1	Keller -killiani Test- Extract of the drug in glacial acetic acid+ few drops of ferric chloride + concentrated sulphuric acid	A reddish- brown colour is formed at the junction of the two layers and upper layer turns bluish green	Presence of cardiac glycosides
2	Legal Test- Extract + pyridine sodium nitroprusside solution and + sodium hydroxide solution.	Change from pink to red colour.	Presence of cardiac glycosides

Table 6: Test for Anthraquinone Glycosides

SNO	Test	Obsevation	Inference
1	Borntrager 's Test- Boil 0.1 gm of the drug with 5 ml of 10 % sulphuric acid filtrate and shake with benzene layer is separated and 10% aqueous ammonia was added and shaken gently	Lower ammonia layer will show red pink colour	Presence of anthraquinone glycosides
2	Modified Borntrager's Test- Boil 0.1 gm of the drug +dilute HCL +5ml of 5% solution of ferric chloride. shake the filtrate with benzene; add dilute ammonia solution to benzene layer	The lower ammonia layer shows pink colour	Presence of anthraquinone glycosides

Table 7: Test for Gums and Mucilages

S.NO	Test	Obsevation	Inference
1	Ruthenium Red Test- Drug + 0.08 Gm of Ruthenium Red +10 ml of 10% solution of lead acetate	Solution turns to red colour	Presence of mucilage
2	Molisch's Test- Aqueous or alcoholic solution of the extract +10% alcoholic solution α -naphthol+ concentrated sulphuric acid along the side of the test tube	A violet ring at the junction of two liquids	Presence of carbohydrates, gums and mucilage

Table 8: Test for proteins and amino acids

SNO	Test	Obsevation	Inference
1	Biuret Test- 2 ml of the extract +2 ml of 10% NaOH solution +2-3 drops of 1%CuSo4 solution were mixed	The appearance of violet or purple colour	Confirms the presence of proteins
2	Ninhydrin Test- 0.5 ml of ninhydrin solution +2 ml of the extract boiled for 2 minutes.	The appearance of blue colour	Confirms the presence of proteins
3	Xanthoproteic Test- 2ml of extract +1 ml of conc. HNO3 boiled and cooled add 40% NaOH solution added drop by drop to it	The appearance of orange colour solution	Confirms the presence of proteins
4	Millon's Test- 2ml of extract +2 ml of million's reagent were boiled and cooled then add few drops of NaNo2	The appearance of red precipitate and red coloured solution	Confirms the presence of proteins

Table 9: Test for tannins and phenolic compounds

SNO	Test	Obsevation	Inference
1	Test with lead acetate- 1 ml of the extract +lead acetate.	White precipitate is formed.	Presence of tannins.
2	Test with Ferric chloride- 5 ml of the extract +5% W/V solution of ferric chloride in 90% alcohol.	Precipitate is formed.	Presence of phenols.
3	Test with gelatine solution- 5 ml of the extract +1% aqueous solution of gelatine +10% sodium chloride.	A white buff precipitate is formed.	Presence of tannins.

Table 10: Tests for flavonoids

SNO	Test	Obsevation	Inference
1	Test with NaOH- The extract was first dissolved with water.it was filtered and the filtrate was treated with sodium hydroxide.	A yellow colour appeared	Confirms the presence of flavonoids.
2	Shinoda Test- Extract +a pinch of magnesium powder was added followed by conc. HCL.	Appearance of pink colour	The presence of flavonoids bioflavonoids

Table 11: Tests for steroids & sterols

SNO	Test	Obsevation	Inference
1	Salkowski's Test- 5 ml of extract in chloroform +conc. Sulphuric acid along with side of the test tubes.	The upper chloroform layer showing a play of colours first form bluish red to gradually violet and lower acid layer showing yellow colour with green fluorescence	The presence of steroids and sterols
2	Liebermann Burchard reagent Test- 5 ml of the extract in chloroform +2 ml of acetic anhydride + 2-3 drops of conc. Sulphuric acid and allow to stand for few minutes	An emerald green colour develops.	The presence of steroids and sterols

Table 12: Test for triterpenoids

SNO	Test	Obsevation	Inference
1	Test with tin and thionyl chloride Extract was dissolved in chloroform + a piece of metallic tin +1 drop of thionyl chloride was added to it.	Pink colour was developing.	The presence of triterpenoids

Table 13: Test for saponins

S.NO	Test	Obsevation	Inference
1	Foam test- 1ml of alcoholic and aqueous extract was diluted separately with distilled water make the volume up to 10 ml, and shaken in a graduated cylinder for 15 minutes and kept aside.	1 cm layer of foam after standing for 30 minutes	The presence of saponins

Table 14: Treatment schedule for anthelmintic activity

Groups	Treatment	Concentration	Purpose
I.	Normal saline (NaCl)	-	To serve as a control
II.	Standard(Fenbendazole)	10% 20% 30%	To serve as a Standard
III.	TEST(Plant extract)	10% 20% 30%	To accesses anthelmintic activity

Table 15: Percentage Yield of Extract

S.NO	Extracts	Colour	Consistency	Yield
1	Ethanolic extract	Dark green	More sticky	7.5%

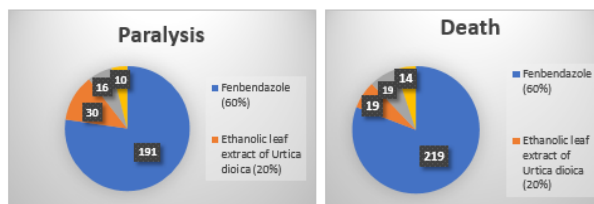
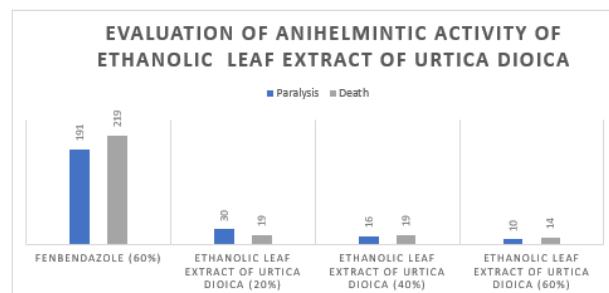
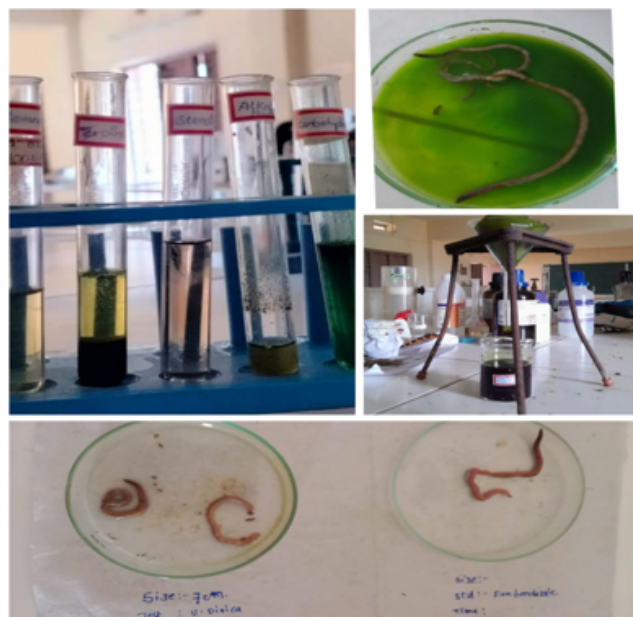
Table 16: Preliminary phytochemical screening

S.No	Class of the compound	Plant part (leaf)	Test performed
1	Alkaloids	+	Dragendroff's test, Mayer's test
2	Carbohydrates	+	Fehling test
3	Glycosides	+	Keller killiani test
4	Proteins and amino acids	+	Xanthoprotic test
5	Flavonoids	+	Ammonia test
6	Saponins	+	With water Na2co3
7	Phenolic compounds and tannins	+	Ferric chloride text

Table 17: Evaluation of anihelminthic activity of aqueous leaf extract of Urtica Dioica

Group	Treatment	Concentration of treatment	Time taken in minutes Sem	
			Paralysis	Death
Group1	Normal saline (NaCl) water	---	---	---
Group2	Fenbendazole	20%	---	---
		40%	---	---
		60%	191± 5.89	219± 3.78
Group3	Ethanolic leaf extract of Urtica dioica	20%	30±	19±
		40%	1.50***	1.93***
		60%	1.12***	0.99***
			10±	14±
			0.72***	0.39****

***p< 0.0001 significant when compared to the control group using Dunnett's method of comparison.



9. Discussion

Helminth infections are among the widespread infections in distressing a huge population of the world: Intestinal worm

infestations are widely prevalent in tropical and subtropical countries and occur where there is poverty and poor sanitation. Soil-transmitted helminth (STH) infections form the most important group of intestinal worms affecting two billion people worldwide and the main species, which infect are *Ascaris lumbricoides*, (roundworms), *Trichuris trichiura*, (whipworm) and *Necator americanus* *Ancylostoma duodenale* (hookworms) According to World Health Organisation (WHO), globally there are 1221 e1472 million cases of Ascariasis, 75001050 million cases of Trichiniasis and 740e1 300 million cases of hookworm infestation.

It was found that various problems like drug resistance occurred to several families of chemical anthelmintic drugs hence the present study is carried to evaluate the antihelminthic potential of some traditional plant of *Urtica dioica* the leaves of *Urtica dioica* is selected to evaluate anthelmintic activity. Aqueous leaf extract of *Urtica dioica* was prepared and anthelmintic activity was evaluated by in-vitro made on earthworms.

The study results showed that phytochemical screening of *Urtica dioica* leaf ethanolic extract contain the following class of phytoconstituents alkaloids, tannins, phenolic compounds proteins amino acids, flavonoids glycosides.

The antihelminthic activity of *Urtica dioica* leaf ethanolic extract (CGLE) was evaluated by In-vitro method on Indian earthworms divided into three groups receiving distilled water, standard and test doses of drug in 20%.40% and 60%. The study results of test and standard are compared by observing time taken for paralysis of worms when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worm neither moved when shaken vigorously nor when dipped in warm water. All the results were expressed as a mean SEM of six worms in each group.

The current study results showed that earthworms (Group II) treated with standard drug fenbendazole at doses of 20% 40%, does not exhibit any paralysis and mortality of worms It is also notified from the present study results that earthworm administered with standard drug at 60% of fenbendazole showed paralysis and mortality of worm after 4 hours of drug administration. A dose dependent of standard drug was from the study result

It was observed from the study results that Group II treated with test drug with 20% and 40% exhibit paralysis and mortality with 30s1:45 354191 and 16x1.06, 19:0.94 minutes. It is also observed from the study results that Group II treated with test drug at doses of 60% also exhibit paralysis and mortality with in 10:0.69, 1440.26 minutes. From the results it was observed that Group II treated with test doses of drug exhibit a significant paralysis and mortality of earthworms when compared to standard group.

The present investigation exhibited that significant anthelmintic activity was showed by test drug when compared to standard drug. The better anthelmintic activity

of leaf extract of *Urtica dioica* compared to standard drug may due to the presence of phytoconstituents in leaf extract which are revealed in phytochemical screening. The plant is reported to contain phytoconstituents like tannins phenolic compounds and alkaloids which might be the reasons for possible and better antihelminthic activity of leaf extract compared to standard drug fenbendazole.

10. Conclusion

In a study assessing the ethanol leaf extract of *Urtica dioica* for its in vitro anti-helminthic activity, it was observed that the extract showed significant effectiveness against helminthic parasites. The extract exhibited potent activity in inhibiting or kill the growth and reproduction of Indian earthworms, indicating its potential as a natural treatment for parasitic infections. The study results when compared to standard drug of fenbendazole, ethanolic extract of *Urtica dioica* possess a good significant anti-helminthic activity compared to standard drug of fenbendazole.

The current research also provides a scope for further expansion for conducting the preclinical and clinical phases of evaluation.

11. Source of Funding

None.


12. Conflict of Interest

None.

References

1. Goodman G. Goodman & Gilman's the Pharmacological. 14th ed. and others, editor;. p. 1121.
2. Hookworm. Available from: <http://en.wikipedia.org/wiki/hookworms>.
3. Report WWH. Conquering suffering enriching humanity 2000; Available from: <https://iris.who.int/handle/10665/41900>.
4. Anokwuru CP, Anyasor GN. Fakoya Okebugwu P. Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants. *Nat Sci*. 2011;9(9):1–7.
5. Peter J, Hotez PJ, Brindley JM, Bethony CH, King EJ, Pearce J. Helminth infection: the great neglected tropical diseases. *J Clin Invest*. 2008;118(4):1311–21.
6. Nematode; Available from: <http://en.wikipedia.org/wiki/Nematode>.
7. Khadse CD, Kakde RB. In vitro anthelmintic activity of Fenugreek s extract against *Pheritima posthuma*. *Int J Res Pharm Sci*. 2010;1(3):267–9.
8. Available from: <http://www.dpd.cdc.gov/dpdx/html/ascariasis.htm>.
9. whipworms. Available from: <http://en.wikipedia.org/wiki/whipworms>.
10. Earthworms. Available from: <http://en.wikipedia.org/wiki/Earthworms>.

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