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## *In-vitro* anthelmintic activity of *Citrus limon* leaf and its phytochemical investigation

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**Abstract**

The crude extract of *Citrus limon* L was evaluated for anthelmintic activity using adult earthworm *Eisenia Foetida*. The prevalence of worm infestations is high in underdeveloped countries due to poor sanitation and lack of health education. *Citrus limon* L. leaf belonging to family Rutaceae is a common plant, native to Asia. Aqueous extract of *Citrus Limon* L leaf was subjected for the preliminary phytochemical analysis and found the presence of alkaloids, tannins, phenolic compounds, carbohydrates, proteins, oils, fats and flavonoids. Anthelmintic activity was performed in 3 different concentrations such as 5mg/ml, 10mg/ml and 20mg/ml. The highest concentration showed the maximum activity. All the extracts exhibited better activity than standard compound Albendazole.

**Keywords:** *Citrus limon* L, *Eisenia Foetida*, phytochemical Analysis, anthelmintic activity, albendazole

**Introduction**

World health organization (WHO) estimated that 80% of the population of developing countries rely on traditional medicines. Mostly plant drugs for their primary health care needs the use of medicinal plant drugs are growing world wide because of its toxicity and allergic manifestation of synthetic drugs hence there is increase in demand towards natural anthelmintics <sup>[1]</sup>. Helminthiasis is one of the most widespread infectious disease affecting mostly children and pregnant women. Around 2 billion people are infected by helminths (*Ascaris lumbricoides*, hookworm, *Trichuris trichiura*, and *Hymenolepsis nana*) especially in the developing countries having poor sanitation <sup>[2]</sup> *Citrus limon* L belongs to family rutaceae is also known as lemon and one of the most important crops of Asia and other parts of south eastern region. Fruit is widely used for culinary, beverages, industrial and medicinal uses.

Traditionally, *Citrus limon* claims as anthelmintic Thus, the present study was design to evaluate the *in-vitro* anthelmintic activity of aqueous extract of *Citrus limon*. Preliminary qualitative phytochemical screening of *C. limon* L leaves revealed the presence of phenolic compounds, terpenoids, flavonoids and alkaloids. These phytochemicals are responsible for biological activity of plant. Considering its various biological activities, anthelmintic activity of leaves extracts was carried out in this experiment. As per the available reports this is the first study of anthelmintic activity of aqueous extracts of *Citrus limon* L leaves collected from Karimnagar district, Telangana, India.

**Materials and Methods**

The plant material was collected from Karimnagar district, Telangana, India.

- The plant parts taken were thoroughly washed to remove foreign matter and they were shade dried at room temperature.
- The complete shade dried leaves were chapped into small pieces and grinded by using a grinder. The powdered leaves were kept in a sterile plastic container and used for experimental study.

**Preparation of plant extract**

The leaf materials of *Citrus* were weighed (250g) and extracted with water (1 lit) at room temperature in a glass containers for 5 days. The each macerated plant materials were stirred from time to time ensures proper extraction. After 5 days, the contents of the containers were filtered through muslin cloth and the filtrates were concentrated under reduced pressure below 50°C, until a soft mass obtained and then preserved in desiccators as labelled CLLE (*Citrus limon* Leaf Extract)

### Anthelmintic activity

Chemicals Albendazole, normal saline were prepared from authorized pharmaceuticals. The solvents and other chemicals used during experimental protocol were of analytical grade.

### Animal

Indian earthworm species *Eisenia foetida* was used due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [6, 7, 8] for evaluating the anthelmintic activity of crude extract using the reference substance for comparison. Earthworms were collected from the water logged areas of soil and identified at the Department of Zoology, Satavahana university, Karimnagar, Telangana. Normal saline solution was used for the assay served as control. The time taken for complete paralysis and death was recorded. External stimuli were applied as ascertain the paralysis time. The time taken by worm to become motionless was considered as paralysis time and lethal time was ascertained by death of motionless worm followed by fading of their body color.

### Phytochemical screening

The extract of *Citrus limon* as CLLE was subjected to chemical investigations to detect the presence of various phytoconstituents in the crude extract. In order to determine the presence of alkaloids, flavonoids, tannins, phenolic compounds, oils, fats, proteins and sugars a preliminary phytochemical study (color reaction) with plant extract and fraction was performed.

**Table 1:** The following chemical tests were performed for extracts.

Active constituents	Various chemical test
	Dragendorff's test
Alkaloids	Mayer's test
	Hager's test
	Wagner's test
Carbohydrates	Molisch's test
	Benedict's test
Tannins	Lead acetate test
	5% Ferric chloride test
Flavonoid glycosides	Shinoda test
Terpenoids	H <sub>2</sub> SO <sub>4</sub> with chloroform
Fats and oils	Sudan red III

### Administration of extracts

The suspension of Aqueous extract of extract of *Citrus limon* in different concentration (5-20mg/ml) were prepared using 5-20 mg extract dissolved in 5ml of saline and final volume is made to 10ml for respective concentration (5mg/ml, 10mg/ml, 20mg/ml) of *Citrus limon* leaves.

### Administration of Albendazole

Albendazole (5, 10, 20mg/ml) was prepared by using 50mg, 100mg, and 200mg powder in 10ml saline as a suspending agent.

### Statistical Analysis

All the results were expressed as mean  $\pm$  S.D. of two animals in each group.

### Anthelmintic potency of *Citrus limon* leaf

Earth worms were divided in three groups of two worms in each group, to assess the anthelmintic activity of aqueous extract of *Citrus limon*.

**Group 1:** Served as control group and worms in this group were placed in distilled water.

**Group 2:** Was served as standard groups of four worms are treated by Albendazole.

**Group 3:** Was act as test group and divided in to three sub groups and each sub groups was treated by aqueous extract at concentration of 5 mg/ml, 10 mg/ml, 20 mg/ml

Observation was made for the time taken to paralyse and death of the individual worms. Mean time for paralysis in minute was noted when worms become motionless and to ascertain death, each worm was frequently applied with external stimuli, which stimulates and induce movement in earth worms. Death was confirmed when no movement in worms even after application of external stimuli.

### Results and Discussions

*Citrus limon* is an annual herb. Majorly the plant used as Antioxidant and Anthelmintic. It reduces inflammations and used in ulcers, conjunctivitis, skin problems. The plant is good in treating dyspepsia and vomiting.

From the phytochemical analysis it was found that the major chemical constituents of leaf were alkaloids, carbohydrates, flavonoids, glycosides, tannins, proteins and oils & fats.

**Table 2:** Show the active constituents various chemical test

Active constituents	Various chemical test	Results
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
Carbohydrates	Molisch's test	+
	Fehling's test	-
	Benedict's test	+
Proteins	Millon's test	+
	Biuret test	-
Tannins	5% Ferric chloride	+
	Lead acetate	+

The *Citrus limon* exists anthelmintic activity due to presence of flavonoids, alkaloids, tannins and phenolic compounds that justify its folkloric use in curing Helminthiasis.

In this anthelmintic activity study, the aqueous leaf extract of *Citrus limon* produce paralysis as well as death of worms. As shown in Table, aqueous leaf extract exhibited anthelmintic activity as compared to standards (i.e., Albendazole). Albendazole caused paralysis and death of the worms. Furthermore, the anthelmintic activity of aqueous leaf extract of *Citrus limon* was comparable with the standard drug.

**Table 3:** *Eisenia foetida* (Earthworms) Time taken Time taken for paralysis for death

S.no	Groups	Concentrations(mg/ml)	<i>Eisenia foetida</i> (Earthworms) Time taken Time taken for paralysis for death in In min min (mean $\pm$ (mean $\pm$ SEM) SEM)	
1	Control (water only)	-	-	-
2	<i>Citrus limon</i> (aqueous extract)	5	34 $\pm$ 1.41	60 $\pm$ 0.70
		10	30 $\pm$ 0.50	50 $\pm$ 0.29
		20	14 $\pm$ 0.26	20 $\pm$ 0.07
3	Albendazole (standard)	10	25 $\pm$ 2.94	60 $\pm$ 3.74

Each value represents mean $\pm$ SEM (N=2)



Fig 1: Control

Fig 2: 5mg/ml

Fig 3: 10mg/ml

Fig 4: 20mg/ml

Fig 5: Albendazole 10mg/ml

### Conclusion

From the phytochemical analysis it was found that the major chemical constituents of leaf were alkaloids, carbohydrates, flavonoids, glycosides, tannins, proteins and oils & fats. It was observed that aqueous extract of leaf possesses significant anthelmintic activity than standard Albendazole. All the values are expressed as mean  $\pm$  SEM (N =2). From the above results, it is concluded that all the aqueous extracts of leaf of *Citrus limon* have potent anthelmintic activity. The concentration (20mg/ml) showed better anthelmintic effect when compared to other concentrations.

This study can be further carried out in the direction that can lead to know the role of secondary metabolites of the plant extracts in the anthelmintic activity. Therefore, further study must be carried out so that the general people can get actual benefits from important medicinal plants like *Citrus limon*.

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