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ANTHELMINTIC ACTIVITY OF *CITRUS LIMON L.* (BURM) PEELS EXTRACT IN INDIAN ADULT EARTHWORM

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Abstract

Citrus limon L. (Burm) popularly known as 'Nimbu' in India belongs to family Rutaceae. The objective of the present work was to evaluate the in-vitro anthelmintic potency of the ethanolic extract of *Citrus limon L* peels using Indian earthworms (*Pheretima posthumad*). The various concentrations (25-100 mg/ml) of the ethanolic extract were tested in-vitro for anthelmintic potency by determination of time of paralysis and time of death of worm. Piperazine citrate (15mg/ml) used as standard. The result of present study indicates that the *Citrus limon L.* potentiate to paralyze earthworm and also caused its death after some time. Thus, the present study demonstrate that the *Citrus limon L.* as an potent anthelmintic has been confirm as the ethanolic extracts of peels displayed activity against the earthworm used in study.

Keywords: *Citrus limon*, Paralysis, death of earthworm.

INTRODUCTION

Helminthiasis or infections with parasitic worms are pathogenic for human beings. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelmintics are the drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues. Most of the existing anthelmintics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea¹. Chemotherapy is the only treatment and effective tool to cure and control helminth infection, as effective vaccines against helminths have not been developed so far. Indiscriminate use of synthetic anthelmintics

can lead to resistance of parasites². Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human³ and could be of value in preventing the development of resistance. Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition. *Citrus limon L.* (Burm) belonging to family *Rutaceae* is an evergreen, aromatic, shrub or small tree^{4,5}. The fruit is used for culinary and nonculinary purposes throughout the world – primarily for its juice, though the pulp and rind (zest) are also used, mainly in cooking and baking⁶. It is an important herbal drug used as antihypertensive, antihyperlipidemic, anticancer, antioxidant and insecticidal remedy⁷. Traditionally, *Citrus limon L.* claim as anthelmintic but scientifically it is not revealed still thus the present study was design to evaluate the in-vitro anthelmintic activity of ethanolic extract of *Citrus limon L.* peels.

MATERIALS AND METHOD

Plant: The fresh fruits of *Citrus limon L.* were collected in the month of October 2010 from its natural habitat at Hadgaon in Nanded region, Maharashtra, India. The plant was authenticated by Dr. Miss. A. Chaturvedi, Post Graduate Teaching Department of Botany, Rashtra Santa Tukadoji Maharaj Nagpur University, Nagpur (Voucher specimen no. 9845).

Experimental animals:

All the experiments were carried out in Indian adult earthworms (*Pheretima posthumad*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity⁸ due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids*, of human beings⁹. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in *vitro*.^{10, 11}

Material: Ethanolic extracts of *Citrus limon L.* peels, Piperazine citrate (GSK. Ltd, Mumbai).

Preparation of Extracts of *Citrus limon L.* peels:

From the collected fruits of *Citrus limon L.* the peels were removed manually and were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using soxhlet extractor. The percent yield of Peel extract was 20% w/w. The extracts were concentrated by evaporation at room temperature and

were used for pharmacological studies¹².Preliminary phytochemical tests of extract was performed by using specific reagents through standard procedures.

Administration of Extract:

The suspension of Ethanolic extract of *Citrus limon L.* different concentration (25-100mg/ml) were prepared by using 0.2% v/v of Tween 20 as a suspending agent and final volume was made to 10 ml for respective concentration of *Citrus limon L.* peels. Six groups of approximately equal size worms consisting of six earthworms individually in each group were released into 10 ml of desired concentration of drug and extracts.

Administration of Piperazine citrate:

Piperazine citrate (15mg/ml) was used prepared by using 0.2% v/v of Tween 20 as a suspending agent.

Experimental Design

Indian adult earthworms (*Pheretima posthumad*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity.

Different concentration (25-100mg/ml) of *Citrus limon L.*ethanolic extract were prepared by using 0.2% v/v of Tween 20 as a suspending agent and final volume was made to 10 ml for respective concentration of *Citrus limon L.* A Piperazine citrate (15mg/ml) was used as standard. Six groups of approximately equal size worms consisting of six earthworms individually in each group were released into 10 ml of desired concentration of drug.

The anthelmintic assay was carried out as per the method of (Ajaiyeoba 2001) with minor modification. The animals were divided into ten y group containing six earthworms each different concentration of extracts and standard drug solution were poured in different Petri dishesh. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when shaken vigorously nor when dipped in warm water (50⁰c), followed with their body colors fading away)¹³.

Statistical Analysis: All the results were expressed as Mean \pm S.E.M. of six animals in each group. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by student's t test. At 95% confidence interval, p values < 0.001 were considered significant¹⁴.

Anthelmintic effect of *Citrus Limon L.* peels in Indian adult earthworms (*Pheretima posthumad*).

For evaluation of anthelmintic activity *Citrus Limon L.* peels extract, group I and II received normal saline and standard Piperazine citrate while group III, IV, V and VI received different concentration of *ethanolic extract* of *Citrus Limon L.* peels respectively

RESULT AND DISCUSSION

The qualitative phytochemical investigation of ethanolic extracts of peels of *Citrus limon L* showed the presence of an array of active chemical constituents including alkaloids, glycosides, Flavonoids and sterols (Table 1).

Table-1: Preliminary phytochemical screening of ethanolic extract of *Citrus Limon L.*

Tests	Ethanolic extract of <i>Citrus limon L.</i> peels
Alkaloids	+
Flavonoids	+
Tannins	-
Terpenoids	-
Steroids	+
Glycosides	+

(+) = Present, (-) = Absent

The results of anthelmintic activity revealed that ethanolic extracts exhibited varying degree of activity against both the worms and caused paralysis followed by death at all tested concentrations. From the above observation made in the Table 2, the extract of *Citrus limon L.* peels was found to show potential anthelmintic activity when compared to standard drug. Ethanolic extract of *Citrus limon L.* peels of highest concentration 100mg/ml showed paralysis at 13.68 min. and death of earthworm at 27.19 min at highest permissible dose which was comparable to standard

Piperazine citrate (Table 2) From the above result, it is clear that ethanolic extract of *Citrus limon L.* peels have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug. It can be concluded that the active constituents responsible for anthelmintic activity present in the *ethanolic extract of peels of Citrus limon L.* Further study need to isolate and revealed the active compound contained in the crude extract of *Citrus limon L.* and to established mechanism(s) of action are required.

Table-2: Anthelmintic Potency of *Citrus limon L.* peels extract.

Treatment	Group	Concentration (mg/ml)	Time of paralysis (min) (Mean±S.E.M)	Time of paralysis (min) (Mean±S.E.M)
Normal Saline (Control)	I	-	-	-
Piperazine citrate	II	15	24.46±0.64	80.69±1.39
<i>Ethanolic extract of Citrus limon L.</i>	III	25	26.47±1.39	61.05±1.17
	IV	50	19.22±0.42	58.45±1.17
	V	75	17.22±0.62	43.58±0.64
	VI	100	13.68±0.22	27.19±0.62

All values represent Mean ± SEM; n=6 in each group. All values are significantly different from reference standard (Piperazine citrate) ***p<0.001. This activity was Concentration dependent. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms.

CONCLUSION

From the results it conclude that, ethanolic extracts of *Citrus limon L.* demonstrate to possess dose dependant anthelmintic activity when compared to Piperazine citrate (Figure 1). From results, peels of *Citrus limon L.* as an

anthelmintic have been confirm as a it displayed potential anthelmintic activity against the worm used in present study. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms. Piperazine citrate acts by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. The possible mechanism of the anthelmintics activity of *Citrus Limon L.* cannot be explained on the basis of our present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis. *Citrus limon L.* may also have activated nicotinic cholinergic receptor in the worms resulting in either persistent depolarization or hyperpolarisation¹⁵. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

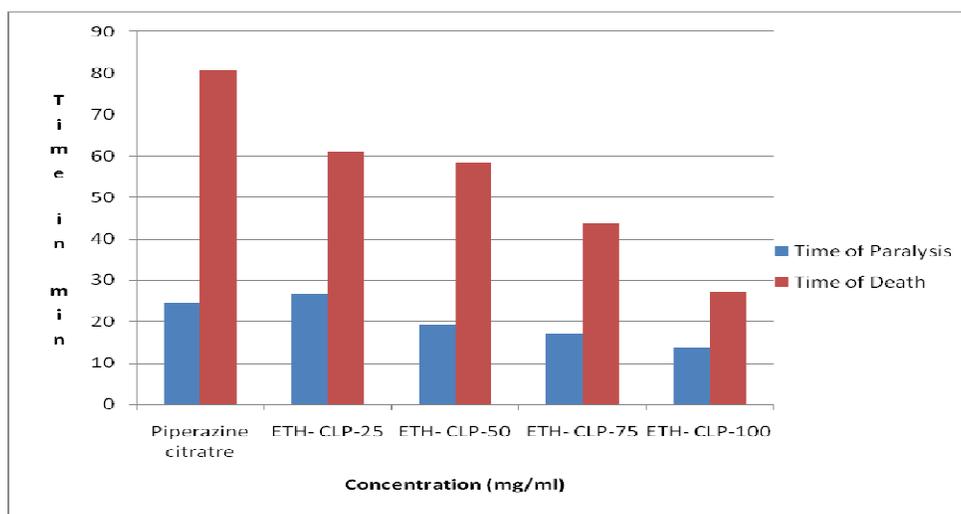


Figure1-Anthelmintic activity of ethanolic extract of *citrus limon L.* peels.

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