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Research Article

Citrus limon L Burmf peel: Potential anthelmintic agent against Indian Earthworm *Eicinia foetida*

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ABSTRACT

Helminthiasis or worm infection is a common infection in human, affecting a large population of the world. Considering this, anthelmintic activity of *Citrus limon* peel was carried out in this experiment. *Citrus limon* belongs to family Rutaceae. Ethyl acetate, ethanol and methanol extracts of *Citrus limon* peel have been evaluated for anthelmintic activity against Indian Earthworm species *Eicinia foetida*. The results reveal a dose dependent increase in activity of the extracts at 5, 10 and 20 mg/ml concentrations. All the extracts exhibited better activity than standard compound Albendazole. Ethanol extract exhibited highest activity among the extract.

Keyword: Anthelmintic activity; *Eicinia Foetida*, Albendazole.

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INTRODUCTION

Helminthiasis is one of the major causes of gastrointestinal disorder¹. It also affects the productivity loss of small ruminant livestock and pets¹. The infections due to worm are a great threat to health and contribute to the prevalence malnutrition, anemia, pneumonia etc². Despite this prevalence of parasitic infections the research on anthelmintic drug is limited³. Anthelmintic are drugs that may act locally to expel worms from the gastrointestinal tract (GIT) or systematically to eradicate adult helminthes or development forms that invade organs and tissues⁴. Most of the existing anthelmintic produces nausea, vomiting head ache and diarrhea. Chemotherapy is the only treatment and effective tool to cure and control helminthes⁴. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites⁵. Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human and could be of value of in preventing the development of resistance⁶.

Citrus limon belongs to family rutaceae is also known as Assam lemon and one of the most important crops of Assam and other parts of north eastern region. Fruit is widely used for culinary, beverages, industrial and medicinal uses. The fruits are sour, rich in vitamin C which strengthens the immune system, acts as an antioxidant and protects cells from radical damage⁷. Infusion of the bark or peel of the fruits is given to relive colic. It is used as antiseptic, antibiotic and antiviral. Traditionally, *Citrus limon* claims as anthelmintic but scientifically it is not revealed still^{8,9}. Thus the present study was design to evaluate the in-vitro anthelmintic activity of different solvent extract of *Citrus limon*. Anthelmintic activity *Citrus limon* fruit empty juice sacs were investigated¹⁰. Comparative study of antioxidants changes including total flavonoid, total phenol and total antioxidant capacity of *Citrus limon* fruits grown at the north of Iran were examined¹¹. Antimicrobial activity of juice in ripens and unripe forms of *C. limon* were studied¹².

Preliminary qualitative phytochemical screening of *C. limon* peel revealed the presence of phenolic compounds, terpenoids, flavonoids and steroids. These phytochemicals are responsible for biological activity of plant. Considering its various biological activities, anthelmintic activity of peel extracts was carried out in this experiment. As per the available reports this is the first study of anthelmintic activity of ethyl acetate, ethanol and methanol extracts of *citrus limon* nonripen peel collected from Biswanath district, Assam, India.

MATERIAL AND METHOD

The plant material was collected from Naduar area, Biswanath chariali district, Assam, India.

Preparation of plant extract: Air shade dried and powdered peel material of *Citrus limon* (50 mg) was treated separately with ethyl acetate, ethanol and methanol (250 ml) by refluxing for 18 hours. Solvents were recovered under reduced pressure to obtain the crude extracts.

Anthelmintic activity

Chemicals

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

Animal

Indian earthworm species *Eicinia foetida* was collected from Krishi Vigyan Kendra, Assam Agricultural University, Tinsukia. All earthworms were of approximately equal size (9-10). The anthelmintic assay was carried out as per the method reported by Ajaycoda *et al* with minor modification¹³. The assay was performed on adult Indian Earth species *Eicinia foetida* due to its anatomical and physiological resemblance

with the intestinal round worm parasite of human. 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 to 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 colour.

Administration of extracts

The suspension of different solvent 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* peel. Five groups of approximately equal size worms consisting of five earthworms individually in each group were released into 10ml of desired concentration of drug and extracts.

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 five animals in each group.

Anthelmintic potency of *citrus limon* peel in Indian earthworms (*Eicinia foetida*)-

For evaluation of anthelmintic activity of *citrus limon* peel group I, II and III received standard Albendazole in normal saline while group IV, V and VI different concentration of ethanol extract, VII, VIII, and IX different concentration of methanol extract and X, XI and XII different concentration of ethyl acetate extract of *citrus limon* peel respectively.

RESULT AND DISCUSSION

Table 1: Anthelmintic potency of *citrus limon* L Burmf peel

Treatment	Group	Concentration (Mg/ml)	Time of paralysis (min)	Time of death (min)
Albendazole	I	5	303 \pm 1.30	180 \pm 1.09
	II	10	248 \pm 1.14	170 \pm 1.30
	III	20	218 \pm 1.67	157 \pm 1.22
Ethanol	IV	5	188 \pm 1	127 \pm 0.83
	V	10	164 \pm 0.70	87 \pm 1.22
	VI	20	142 \pm 0.83	64 \pm 1.30
Methanol	VII	5	203 \pm 1.51	132 \pm 1.34
	VII	10	186 \pm 1.30	129 \pm 0.70
	IX	20	158 \pm 1.64	81 \pm 1.30
Ethyl acetate	X	5	202 \pm 1.34	166 \pm 1.51
	XI	10	178 \pm 0.83	127 \pm 1.30
	XII	20	182 \pm 1.09	137 \pm 0.83

All values represents Mean \pm S.D; n=5 in each group. All values are significantly different from reference standard (Albendazole). 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.

From the above result, it is clear that all extracts exhibited better result than standard drug albendazol. Ethanol extract of *Citrus limon* peel have significant anthelmintic activity in dose dependent manner when compared with standard as well as other extracts. It can be concluded that the active constituents responsible for anthelmintic activity present in these extracts of *Citrus limon* peel. Further study is required to isolate active compound/s from the crude extract of *Citrus limon* and to establish the mechanism of action.

CONCLUSION

Citrus limon peel extracts are found to do superior anthelmintic agent than the standard compound albendazole. So it can be concluded that *C. limon* peel extracts have great potential as anthelmintic agent. It can be evaluated as a potential source of natural bioactive chemicals. These studies correlate relationship of these secondary metabolites to possible biological activities and evaluate *Citrus limon* as a potential source of natural bioactive chemicals.

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