

Anthelmintic Activity of *Citrus medica* L. leaves in Indian Adult Earthworm

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Abstract: *Citrus medica* L. popularly known as 'Bara nimbu' in India belongs to family Rutaceae. The objective of the present work was to evaluate the in-vitro anthelmintic potency of the petroleum ether extract of *Citrus medica* leaves using Indian earthworms (*Pheretima posthumad*). The various concentrations (20-80 mg/ml) of the petroleum ether 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 medica* L. potentiate to paralyze earthworm and also caused its death after some time. The shortest time of paralysis and time of death was observed at higher dose (80 mg/ml) of petroleum ether extract was found to 30.86 min. Thus, the present study demonstrate that the *Citrus medica* L. as an anthelmintic has been confirm as the petroleum ether extracts of leaves displayed activity against the earthworm used in study.

Keywords- *Citrus medica*, 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 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 diarrhoea¹. 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 medica* L.,

commonly known as citron, is a small tree, having large fruit (20-22.5 cm. long) resembling pineapple in shape. It is reported that *C. medica* essential oil showed fungitoxicity against some fungi. *C. medica* is relevant to treatment of diabetes and alzheimer's disease.^{4, 5} The *Citrus medica* L. also have an anti-inflammatory, antihistamine and diuretic action and can cause dilatation of the coronaries. Twenty-seven and twenty nine components were identified in the leaf and peel oils, respectively. Limonene was the major constituent in the oil of leaf and peel.^{6,7} Traditionally, *Citrus medica* L. claim as anthelmintic but scientifically it is not reveled still thus the present study was design to evaluate the in-vitro anthelmintic activity of petroleum ether extract of *Citrus medica* L. leaves.

Material and Method

Plant: The fresh leaves of *Citrus medica* 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. 9844).

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 beings.⁹ Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*.^{10, 11}

Material: Petroleum ether extracts of *Citrus medica L.* leaves, Piperazine citrate (GSK. Ltd, Mumbai).

Preparation of Extracts of *Citrus medica L.* leaves:

The collected leaves of *Citrus medica L.* were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using maceration. The percent yield of Petroleum ether extract was 20.8% w/w. The extracts were concentrated by evaporation at room temperature and were used for pharmacological studies.¹² Preliminary phytochemical tests of extract was performed by using specific reagents through standard procedures.

Administration of Extract:

The suspension of Petroleum ether extract of *Citrus medica L.* different concentration (20-80mg/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 medica L.* leaves. 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 (20-80mg/ml) of *Citrus medica L.* petroleum ether 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 medica 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 *et.al*, 2001)¹³ 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 significant.¹⁴

Anthelmintic effect of *Citrus medica L.* leaves in Indian adult earthworms (*Pheretima posthumad*).

For evaluation of anthelmintic activity *Citrus medica L.* leaves, group I and II received normal saline and standard Piperazine citrate while group III, IV, V and VI recieved different concentration of *petroleum ether extract* of *Citrus medica L.* leaves respectively.

Table 1: Preliminary phytochemical screening of petroleum ether extract of *C. medica*

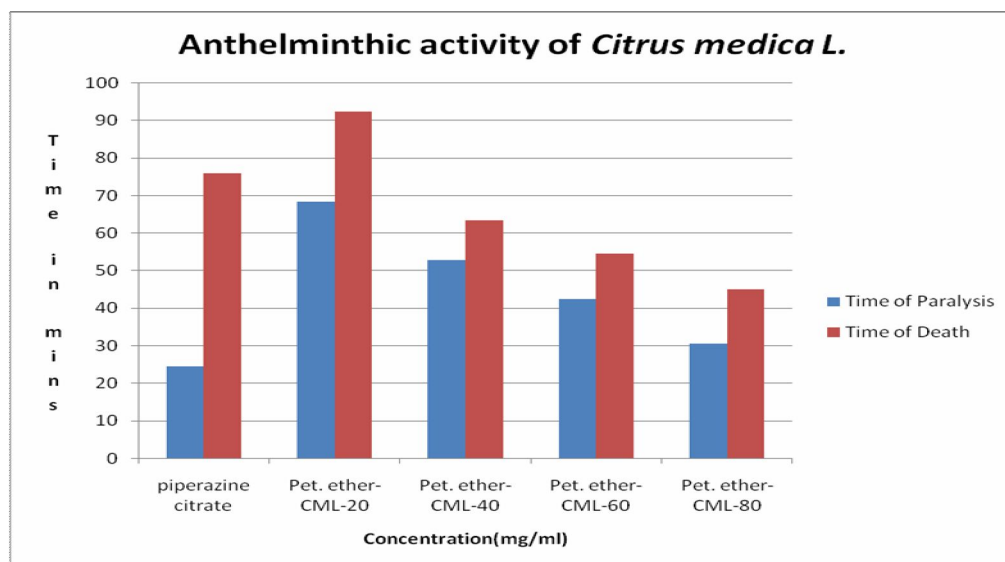
Tests	Petroleum ether extract of <i>Citrus medica L.</i>
Alkaloids	+
Flavonoids	+
Tannins	-
Terpenoids	-
Steroids	+
Glycosides	+

(+) = Present, (-) = Absent

Table 2: Anthelmintic Potency of *Citrus medica L.* leaves

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	76±1.11
<i>Petroleum ether extract of Citrus medica L.</i>	III	20	68.41±0.53	92.53±0.63
	IV	40	52.85±0.41	63.35±0.43
	V	60	42.47±0.43	54.64±0.74
	VI	80	30.42±0.82	45.04±0.48

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.



Pet. ether-CML- Petroleum ether extract of *Citrus medica L.*

Figure1-Anthelmintic activity of petroleum ether extract of *Citrus medica L.* leaves.

Result

The qualitative phytochemical investigation of petroleum ether extracts of *Citrus medica L.* showed the presence of an array of active chemical constituents including alkaloids, glycosides, Flavonoids and sterols (Table 1). The results of anthelmintic activity revealed that petroleum ether extracts exhibited varying degree of activity against 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 medica L.* leaves was found to show anthelmintic activity when compared to standard drug. Petroleum ether extract of *Citrus medica L.* leaves of highest concentration

80mg/ml showed paralysis at 30.42 min. *Petroleum ether extract* of *Citrus medica L.* showed death of earthworm at 45.04 min at highest permissible dose (80mg/ml) which was comparable to standard Piperazine citrate (Table 2) From the above result, it is clear that petroleum ether extract of *Citrus medica L.* leaves 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 *petroleum ether extract* of *Citrus medica L.* leaves. Further study need to isolate and revealed the active compound contained in the crude extract of

Citrus medica L. and to established mechanism(s) of action are required.

Discussion

From the results it conclude that, petroleum ether extracts of *Citrus medica L.* demonstrate to possess dose dependant anthelmintic activity when compared to Piperazine citrate. From results, *Citrus medica L.* as an anthelmintic have been confirm as a it displayed 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 medica 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 medica L.* may also have activated nicotinic cholinergic receptor in the worms resulting in either persistent depolarization or hyperpolarisation¹⁵. There need further studies to identify the active constituent responsible for anthelmintic activity.

References

1. Goodman LS. Gilman A. The Pharmacological basis of Therapeutics.10th Edn. Mcgraw Hill Medical Publishing Division, New York, 2001, 1121
2. Singh D, Swarnkar CP, Khan FA. Anthelmintic resistance in gastrointestinal nematodes in livestock in India, J Vet Parasit ,2002, 16,115-130.
3. Chopra RN, Nayer SC, Chopra IC. Glossary of Indian medicinal plants, CSIR, NewDelhi, 1956, 160.
4. A.C. Andrews, Acclimatization of citrus fruits in the Mediterranean region,Agricultural History, 1961, 1, 35.
5. Z. Fleisher and A. Fleisher, Water-Soluble Fractions of the Essential Oils" Perfumer and Flavorist., 1996, 21, 6, 11.
6. The citrus plant,. in: Citrus. Ciba-Geigy Agrochemicals Tech. Monogr.4. Ciba-Geigy Ltd., Basle, Switzerland, 1975, p.6-13
7. Hesperides. A history of the culture and use of citrus fruits, p.371. John Bale, Sons and Curnow, 1938, London, England.
8. Hammond J.A.Fielding D.and Bishop S.C., Prospects for plant anthelmintics in tropical veterinary medicines, Vet. Res. Coom., 1997, 21,213-228.
9. Vidyarthi RD. *A Text Book of Zoology*, 14th Edn., S. Chand and Co, New Delhi, 1967.
10. Sollmann T. Anthelmintics: Their efficiency as tested on earthworms. *J. Pharmacol. Exp. Ther.* 12, 129-170
11. Jain ML and Jain SR. Therapeutic utility of *Ocimum basilicum* var. *album*. *Planta. Med.* 1972,22,66-70.
12. Sharangouda, Patil S.B., Estrogenic activity of petroleum ether extract of *Citrus medica L.* on immature albino rats,Int. Jr. Green Pharm., 2007, 2: 91-94.
13. Ajaiyeoba EO, Onocha PA and Olarenwaju OT. *In vitro* anthelmintic properties of *Buchholzia coriacea* and *Gynandropsis gynandra* extracts. *Pharma. Biol.*, 2001, 39, 217-220.
14. Armitage P. Statistical methods in Medical Research. 1st Edn. Blackwell scientific publication, New York, 1971, 217-220.
15. Tripathi KD. Essential of Medical Pharmacology. 4th Edn. Jaypee Publication, New Delhi, 2006, 816-817p
