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A STUDY ON IN VITRO ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACTS OF LEAVES *CITRUS AURANTIFOLIA SWINGLE* AGAINST *PHERITIMA POSTHUMA*

Asha Jadhav*, Sandip Patil, Shahin Inamdar

Dept. of Pharmacology, Adarsh College of Pharmacy, Vita, Sangli, Maharashtra 415311, India

***Corresponding Author**

Asha Sambhaji Jadhav

Adarsh College of Pharmacy,
Kundal Road, Bhavaninagar, Vita.

Tal-Khanapur, Did- Sangli 415311.

Mobile No. 9970530289

E mail address: ashajadhav89@gmail.com

INTRODUCTION

Helminthiasis or worm infestation is one of the most prevalent disease and one of the most serious problems in the world. It contributes to malnutrition anemia, eosinophilia and pneumonia^[1, 2]. A number of plants have been tested for their anthelmintic efficacy^[3]. *Citrus aurantifolia swingle* is known as lemon plant Rutaceae. It belongs to family Rutaceae^[4]. Due to limited availability and affordability of pharmaceutical medicines, species of higher plants are used for the treatment of helminthiasis. According to W.H.O. more than 80% of the world's population relies on medicinal plants^[1, 3]. *Citrus aurantifolia swingle* is an important medicinal herb found as a weed throughout India. It is known by different names such as Nimbuka (Sanskrit), Khatta nimbu (Hindi) and Lemon plant (English). *Citrus aurantifolia swingle* is an evergreen tree which can reach up to 5m high. The leaves are medium sized measuring 6-9cm long, ovate, bluntly pointed at tips, rounded to cunate at the base. The flowers are white, solitary or in a short



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racemes, small and fragrant. The fruits are yellow when ripe, globose measuring 4-5 cm in diameter with thinner rind and very sour. ^[3&4] This plant was reported to contain saponins, Tanins, flavonoids and alkaloids. ^[5] Saponin A was identified as D-glucuronic acid and Saponin B was identified as β -D-galactopyranosyl ester of D-glucuronic acid. There were no reports on the anthelmintic activity of *Citrus aurantifolia* S. leaves extracts. This promoted us to investigate and compare the anthelmintic activity of ethanolic extracts of leaves of *Citrus aurantifolia* S. with respect to standard. Traditionally, the plant is used in asthma. It has various medicinal properties reported such as antimicrobial, antiplatelet, antifertility, immunomodulatory, cytotoxic activity, antithyroid, and antiobesity ^[5&6].

MATERIALS METHODS CHEMICALS AND DRUGS

Methanol, Mebendazole (Talent health care Ltd). All the chemicals used were analytical grade.

Collection of Plant Material

The leaves material *Citrus aurantifolia* Swingle were procured from Nerli (Kadegaon). Plant was authenticated by Dr. S. M. Shendage, botany department, Balwant college of Vita. A voucher specimen (Herbarium No. SBI 001) was deposited at Herbarium department of botany, Balwant college of Vita. ^[6]

Preparation of Extracts

The ethanolic extraction was performed by using Soxhlet apparatus. The resultant extract was evaporated to dryness under reduced pressure in Rotary vacuum evaporator. The concentrated extract was liquated in amber-colored bottles and kept in desiccators for further use.

Preliminary Phytochemical Testing

Various phytochemical tests for tannins, saponins, carbohydrates, glycosides, phenols and flavonoids were conducted for both methanolic and aqueous extracts ^[7].



Experimental Design

Indian adult earthworms (*Pheretimaposthuma*) were used to evaluate anthelmintic activity. The earthworms were collected from the local supplier and were authenticated by Department of Zoology, Balwant College, Vita (India). Worms were washed with normal saline to remove all fecal matter. The average size of earthworms was 6-8 cm in length and 0.2-0.3 cm in width was used during experimental procedure. The worms were acclimatized to the laboratory condition before experimentation. Mebendazole was selected as a standard drug ^[8].

Anthelmintic Activity

The anthelmintic activity was evaluated on adult Indian earthworms by Mathew *et.al* method ^[10]. For preliminary evaluation of anthelmintic activity test samples of the extract was prepared at the Concentration of 2.5, 5, 10, 20 mg/ml in Tween 20 (1%) solution diluted with normal saline and 6 worms *Pheretima posthuma* of 6-8cm were placed in petridish containing 30 ml of above test solutions of extracts. Mebendazole (2.5, 5, 10, 20 mg/ml) was used as reference standard and normal saline with Tween 20 (1%) is used as negative control. All the test solutions and standard solutions were prepared freshly before starting the experiment. Observations are made for the time taken for paralysis when movement was lost or no movement. Worms should not relieve even in normal saline. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water and fading of color of worms^[9].

RESULTS AND DISCUSSION

Different concentrations (2.5, 5, 10, 20 mg/ml) of ethanolic extract of leaves of *Citrus aurantifolia swingle* were evaluated for *in-vitro* anthelmintic activity. The percentage yield of ethanolic extract was obtained 10.5 & 7.3% w/w respectively. Preliminary phytochemical screening of extract revealed the presence of saponins, carbohydrates, glycosides, phenols & flavonoids and absence of tannins (Table 1).Anatomical and Physiological resemblances with human intestinal round worm parasite of human beings.

Table 1. Preliminary Phytochemical Screening of *Citrus aurantifolia* swingle ethanolic extracts of leaves.

| <i>Sr. No.</i> | <i>Phytochemical Test</i> | <i>E. C. A.</i> |
|----------------|---------------------------|-----------------|
| 1. | Test for alkaloids | ++ |
| 2. | Test for carbohydrates | + |
| 3. | Test for Steroids | + |
| 4. | Test for Flavonoids | ++ |
| 5. | Test for Glycosides | ++ |
| 6. | Test for saponins | + |
| 7. | Test for Trepenes | + |

ECA: *Ethanolic extract of leaves of Citrus aurantifolia swingle* (+) indicate presence of test.
(-) indicate absence of test.

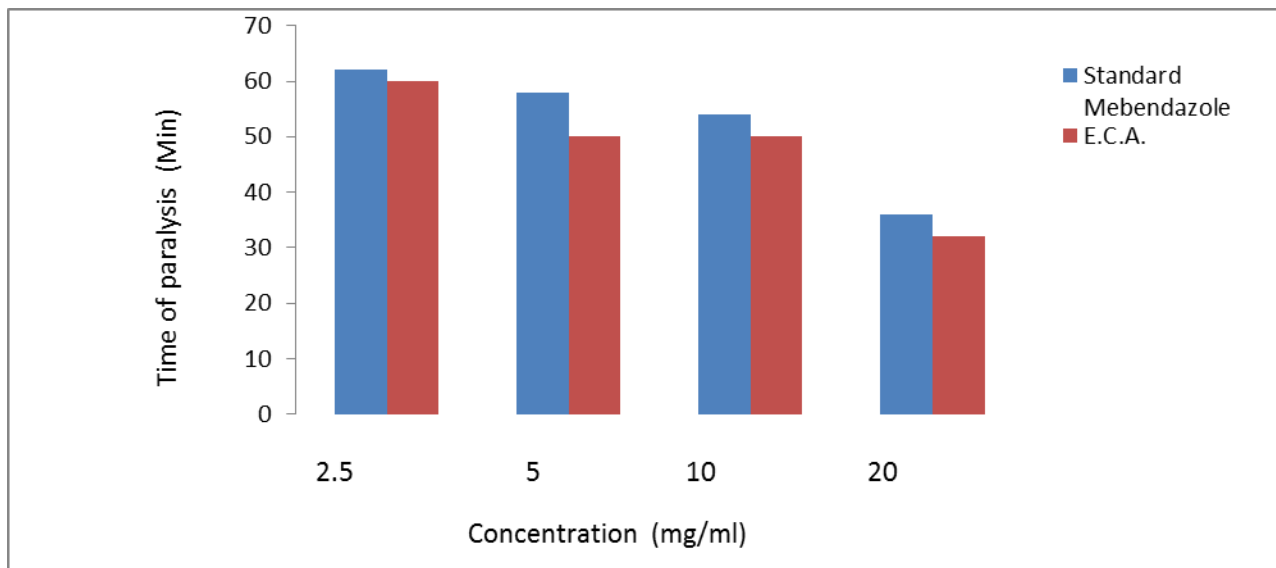
The results in the Table-2 depict the time taken for paralysis and the time taken for death after treating with the test substances.

Table 2. Anthelmintic activity (Time of paralysis & death) of leaves *Citrus aurantifolia swingle*

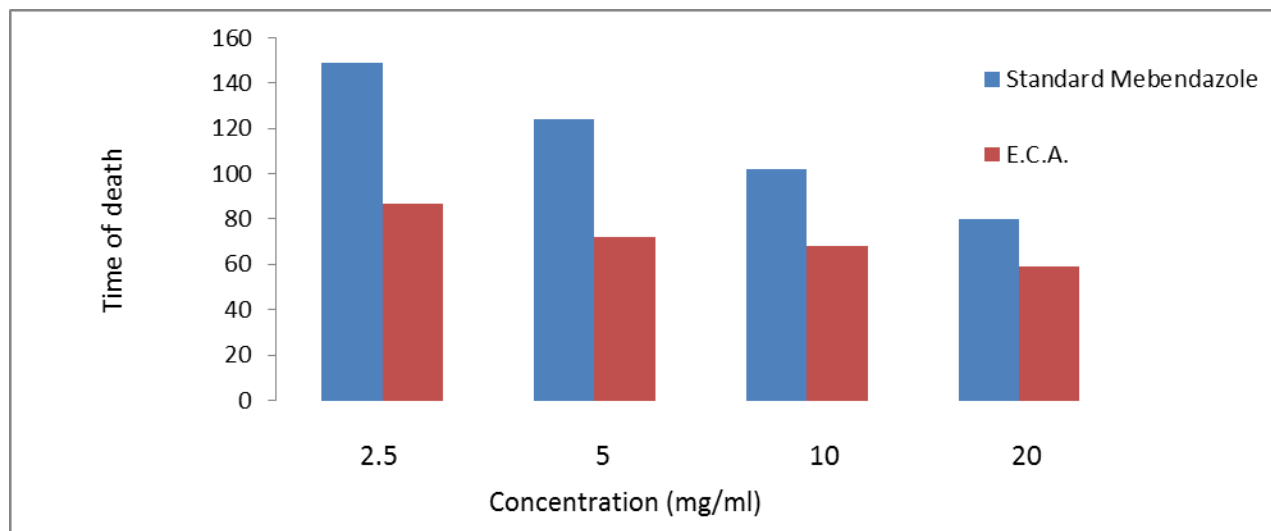
| Treatment | Concentration (mg/ml) | <i>Pheritima posthuma</i> | |
|----------------------------|--------------------------|---------------------------|--------------------|
| | | Time of paralysis (Min) | Time of Death(Min) |
| Control (Normal saline) | - | - | - |
| Standard Mebendazole | 2.5 | 62 | 149 |
| | 5 | 58 | 124 |
| | 10 | 54 | 102 |
| | 20 | 36 | 80 |

| | | | |
|--------|-----|----|----|
| E.C.A. | 2.5 | 60 | 87 |
| | 5 | 52 | 72 |
| | 10 | 50 | 68 |
| | 20 | 32 | 59 |

Graph: 1. Effect of different concentrations of ethanolic extract on time of paralysis of worms.



Graph: 2. Effect of different concentrations of ethanolic extract on time of death of worms.



Anthelmintic activities of extract was evaluated. Extract at a concentration of 20 mg/ml, produced paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death. The ethanolic extract of *Citrus aurantifolia swingle* of leaves extract at normal concentration only showed good anthelmintic activity and this is compared with the effect produced by the reference standard drug, Mebendazole. The results in Table-2 indicate that ethanolic extract obtained from the leaves of *Citrus aurantifolia swingle* is active against the *Pheretima posthuma* tested. It is worth to mention that the leaves ethanolic extract is comparatively more active than Mebendazole against *Pheretima posthuma*. These findings support the use of *Citrus aurantifolia swingle* as anthelmintics in the traditional medicine. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms.



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CONCLUSION

In conclusion, it is revealed that the Ethanolic extract obtained from the leaves of *Citrus aurantifolia* possess anthelmintic activity, when compared with standard drug, Mebendazole. In order to confirm the above results, the *in vivo* studies have to be conducted.

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