



COMPARATIVE STUDIES ON ANTHELMINTIC ACTIVITY OF *CITRUS MEDICA L.* AND *OCIMUM BASILICUM L.*

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Accepted on: 18-02-2011; Finalized on: 28-04-2011.

ABSTRACT

Methanolic extracts of *Citrus medica L.* and *Ocimum basilicum L.* were taken for anthelmintic activity against Indian adult earthworm *Pheretima posthuma*. Various concentrations of both extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/ml) was used as a reference standard and distilled water as a control group. Dose dependent activity was observed in both plant extracts but *Citrus medica L.* shows more activity as compared to *Ocimum basilicum*.

Keywords: *Citrus medica L.*, *Ocimum basilicum L.*, Anthelmintic activity, Piperazine citrate.

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 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 resistance⁴.

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.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}

Sweet basil (*O. basilicum L.*) is known locally as Ofesleuen or reyhano, is native to Asia and is widely grown as an ornamental or field crop from seed in the Mediterranean countries, including Turkey^{8,9}. Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactagogue, stomachic, and tonic agents.¹⁰⁻¹²

MATERIALS AND METHODS

Plant: The fresh leaves of *Citrus medica L.* and *Ocimum basilicum L.* were collected in the month of October 2010 from its natural habitat at Pusad in Yavatmal region, Maharashtra, India.

Experimental animals: All the experiments were carried out in Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity¹³ due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids*, of human beings.¹⁴ Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in *vitro*.^{15,16}

Material: Methanolic extracts of *Lawsonia inermis L.* leaves, Piperazine citrate (GSK. Ltd, Mumbai).

Extract Preparation: The collected materials were washed thoroughly in water, chopped, air dried for a week at 35-40°C and pulverized in electric grinder and exhaustively extracted successively in soxhlet apparatus, using methanol. The extracts were concentrated under reduced pressure and were then made to powder.

Administration of Extract: The suspension of extract of *Citrus medica L.* and *Ocimum Basilicum* of different concentrations (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. Ten 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.* and *Ocimum Basilicum* leaves extracts 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 a Piperazine citrate (15mg/ml) was used as standard. The anthelmintic assay was carried out as per the method of 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 dish.

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).¹⁷

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.¹⁸

Table 1: Comparative Anthelmintic Activity of *Citrus medica L.* and *Ocimum basilicum*

Methanolic extract of leaves	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
<i>Citrus medica L.</i>	20	78.41 \pm 0.53	92.53 \pm 0.63
	40	57.85 \pm 0.51	73.35 \pm 0.47
	60	49.47 \pm 0.33	54.64 \pm 0.74
	80	30.55 \pm 0.47	49.04 \pm 0.84
<i>Ocimum basilicum L.</i>	20	168.42 \pm 2.13	322.5 \pm 2.25
	40	127.59 \pm 3.07	245.5 \pm 5.20
	60	93.05 \pm 1.60	192.14 \pm 3.18
	80	55.94 \pm 1.67	118.50 \pm 1.6
Piperazine citrate	15	24.46 \pm 0.64	76 \pm 1.11

All values represent Mean \pm SEM; n=6 in each group. 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.

RESULTS AND DISCUSSION

From results, *Citrus medica L.* leaves. shows more anthelmintic as compared with *Ocimum basilicum L.* 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. From the results it is observed that *Citrus medica L.* leaves extract shown potent anthelmintic activity while the *Ocimum basilicum* leaf extract has taken long time for death of worms (Table1). *Citrus medica L.* is showing paralysis in 30.55 min at higher concentration while death is comparable with that of piperazine citrate as death of worms was observed at 45 min. *Ocimum basilicum* was taken 55.59 min to bring paralysis and 76 min to bring death of worms. Future scope involves need of isolation of phytoconstituents responsible for activity.

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