

## Anthelmintic activity of *Lawsonia inermis* L. Leaves in Indian Adult Earthworm

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### Abstract

*Lawsonia inermis* L. leaves, popularly known as Henna or Mehendi in India belongs to family Lythraceae. The objective of the present work was to evaluate the in-vitro anthelmintic potency of the petroleum ether extract of *Lawsonia inermis* leaves using Indian earthworms (*Pheretima posthumad*). The various concentrations (25, 50, and 75 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 *Lawsonia inermis* L. potentiate to paralyze earthworm and also caused its death after some time. The shortest time of paralysis was observed at higher dose (75 mg/ml) of petroleum ether extract was found to 30.86 min. Thus, the present study demonstrate that the *Lawsonia inermis* L. as an anthelmintic has been confirm as the petroleum ether extracts of leaves displayed activity against the earthworm used in study

**Key Words:** *Lawsonia inermis*, 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. Anthelminthics 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 anthelminthics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhoea<sup>1</sup>. 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 parasites<sup>2</sup>. Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human<sup>3</sup> and could be of value in preventing the development of resistance<sup>4</sup>. *Lawsonia inermis* L. commonly known as Henna or Mehendi and abundantly available in tropical and subtropical areas. Ancient history of India describes its diverse uses and also plays appreciable role in Ayurvedic or natural herbal medicines.<sup>5</sup> The plant has been reported to have analgesic, hypoglycemic, hepatoprotective,

immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, nitrotrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.<sup>6,7</sup>

Traditionally, *Lawsonia inermis* 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 petroleum ether extract of *Lawsonia inermis* L. leaves.

### MATERIAL AND METHOD

**Plant:** The fresh leaves of *Lawsonia inermis* L. were collected in the month of October 2010 from its natural habitat at Hadgaon in Nanded region, Maharashtra, India.

### 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 activity<sup>8</sup> due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids*, of human beings.<sup>9</sup> Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro.<sup>10,11</sup>

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

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### Preparation of Extracts of *Lawsonia inermis* L.leaves:

The collected leaves of *Lawsonia inermis* 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 used for pharmacological studies.

### Administration of Extract:

The suspension of *petroleum ether extract* of *Lawsonia inermis* L. different concentrations (25-75mg/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 *Lawsonia inermis* L. leaves. Five 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 (25-75mg/ml) of *Lawsonia inermis 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 *Lawsonia inermis*. A Piperazine citrate (15mg/ml) was used as standard. The anthelmintic assay was carried out as per the method of (Ajaiyeoba et.al, 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 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 (50°C), followed with their body colors fading away).<sup>13</sup>

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.<sup>14</sup>

### Anthelmintic effect of *Lawsonia inermis* L.leaves in Indian adult earthworms (*Pheretima posthumad*):

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

### RESULT AND DISCUSSION

From the observation made in the Table 1, the *petroleum ether extract* of *Lawsonia inermis* L.leaves was found to show anthelmintic activity when compared to standard drug. *Petroleum ether extract* of *Lawsonia inermis* L. leaves of concentration 25, 50 and 75 mg/ml showed paralysis at 62.57, 54.13 and 30.86 min and death found to 185.20, 143.88 and 124.68 min respectively. *Petroleum ether extract* of *Lawsonia inermis* L.showed paralysis of earthworm at 30.86 min at highest permissible dose (75mg/ml) which was comparable to standard Piperazine citrate (Table 1) From the above result, it is clear that *petroleum ether extract* of *Lawsonia inermis* 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 *Lawsonia inermis* L.leaves. Further study need to isolate and revealed the active compound contained in the crude extract of *Lawsonia inermis* L. and to establish mechanism(s) of action are required.

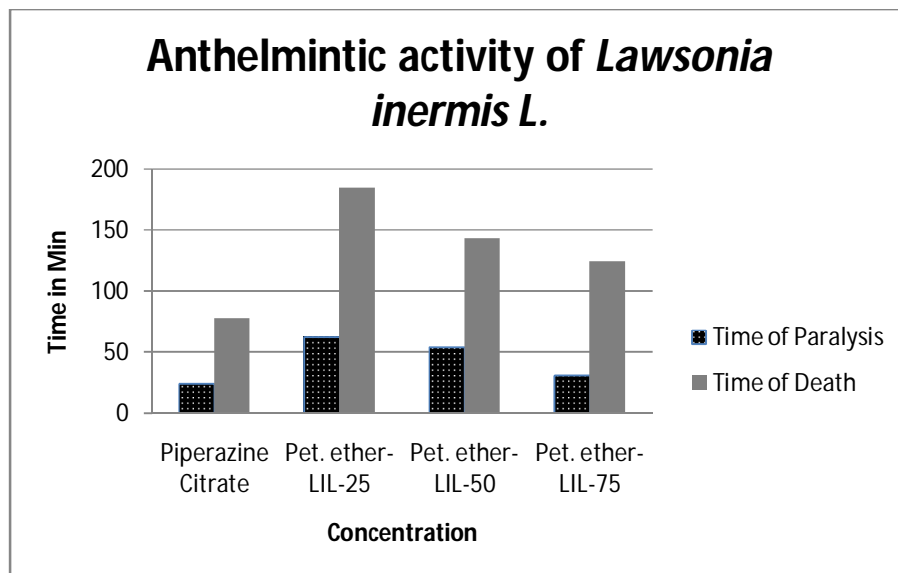
### CONCLUSION

From the results it conclude that, *petroleum ether extracts* of *Lawsonia inermis* L. demonstrate to possess dose dependant anthelmintic activity when compared to Piperazine citrate. From results, *Lawsonia inermis* 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. The possible mechanism of the anthelmintics activity of *Lawsonia inermis* 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. *Lawsonia inermis* L.may also have activated nicotinic cholinergic receptor in the worms resulting in either persistent depolarization or hyperpolarisation.<sup>15</sup>

Table 1: Anthelmintic Potency of *Lawsonia inermis* 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	24.18±0.70	78.21±0.23
<i>Petroleum ether extract of Lawsonia inermis</i>	III	250	62.57±0.63***	185.20±1.41***
	IV	500	54.13±0.63***	143.88±0.68***
	V	750	30.86±0.51***	124.68±1.32***

All values represent Mean ± 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.



Pet. ether LIL- Petroleum ether extract of *Lawsonia inermis* L. leaves

**Fig1- Anthelmintic activity of *Lawsonia inermis***

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