

## PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND IN-VITRO ANTHELMINTIC ACTIVITY OF *LAWSONIA INERMIS* LEAVES

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### ABSTRACT

The present research work deals with phytochemical investigation and in-vitro anthelmintic activity of *lawsonia inermis* leaves on Indian earthworms, *Pheretima posthuma* having physiological and anatomical similarity with intestinal helminthes. Standardization of crude drugs isolated from plant plays a vital role in identification of the quality and purity of drugs. Now a day parasitic (helminthes) infections are growing rapidly worldwide and numerous synthetic antihelmintic drugs are in use for the treatment of helminthiasis but they possess some allergic manifestations as well as adverse effects, in order to overcome that effects natural anthelmintic agents are required in present scenario. This research observed and highlights that ethanolic extracts of *lawsonia inermis* leaves had the highest number of phytochemicals compared to other solvent extracts. Hence, ethanolic extracts of *lawsonia inermis* leaves holds the greatest potential to treat various human diseases and has profound medical applicability. The adult worms are collected and cleaned in normal saline solution before they were placed in to respective formulation. To observe anthelmintic activity, all the investigations were carried out by ethanol, acetone and aqueous extract with different concentrations of 10, 25, 50mg/ml. A significant activity was observed at highest concentrations of 50mg/ml of ethanolic extract and compared with albendazole (10, 25, 50mg/ml) as standard.

**KEYWORDS:** *lawsonia inermis* leaves, *Pheretima posthuma*, Albendazole.

### INTRODUCTION

Standardization of crude drugs isolated from plant plays a vital role in identification of the quality and purity of drugs. Plants are very important in medicinal field; they have similar properties as conventional pharmaceutical drugs. Plant medicines are safer due to their lower chances of side effects and also better compatibility to humans. Medicinal plants are used a wide range for the pharmaceutical products to herbal medicine preparations. Now a day, medicinal plants are considered as potential sources for the preparation of new drugs. The use of medicinal plant is growing worldwide because of the increasing toxicity and allergic manifestations of synthetic drug.<sup>[1-8]</sup> Globally around 24% population is suffering with parasitic infections and they are intended to use synthetic drugs for the treatment but adverse effects of synthetic drugs along with increasing toxicity there is a need to develop natural drugs for treatment of helminthiasis. Thus we can save our future generations from such major problems without any adverse effects. This helminthiasis is caused due to maintenance of poor hygienic conditions, improper waste disposal, eating uncooked meat or infected animals and touching infected surfaces.<sup>[9-18]</sup> Infection is most common in urban places

and severity is high in children so not only use of drugs can cure helminthiasis but there is a need to follow precautions and preventive measures. Observe anthelmintic activity on adult Indian earthworms, *Pheretima posthuma* having physiological and anatomical similarity with intestinal helminthes of human beings.<sup>[19-21]</sup> *Pheretima posthuma* were used for estimation of anthelmintic activity on *lawsonia inermis* leaves because of their easy availability. The present research work deals with phytochemical investigation and in-vitro anthelmintic activity of *lawsonia inermis* leaves.

### MATERIALS AND METHODS

#### COLLECTION OF PLANT MATERIAL

*Lawsonia inermis* leaves were collected from Sathupally, washed with freshly prepared distilled water using simple distillation technique to remove impurities and dust present in surface of leaves and then leaves are air dried on a clean sterile paper to remove excess moisture content and then shade dried for 2 days and finely pulverised using mechanical mixer.

### EXTRACTION PROCEDURE

*Lawsonia inermis* leaves were washed thoroughly with distilled water and chopped into minute pieces and air dried at room temperature for 48 hours. The dried leaves were crushed using mixer into fine powder and stored at room temperature. 20 grams of leaf powder is obtained from 100 grams of leaves and macerate the leaves using desirable solvents ethanol, acetone and aqueous in specified ratio, with frequent agitation so that entire constituents are solubilised into solvents and filter the contents using standard methods and the extracted extracts are stored in air tight containers.

### PREPARATION OF WORMS

Adult India earth worms, *Pheretima posthuma* are collected from the water logged areas of soil at the ground of Mother Teresa pharmacy college, Sathupally. They are placed into a clean sterilised beaker and worms are washed thoroughly with distilled water and placed in sterilised petriplates for further activity determination. *Pheretima posthuma* are very important and play a crucial role as soil invertebrates that promote soil fertility. Earthworm feeding and burrowing activities improve

breakdown of organic matter, improve aeration, release nutrients, aggregation of soil and improve aeration. The average sizes of *Pheretima posthuma* were 8-12 cm with weight 0.8-2.30g were used for all experimental work.

### RESULTS AND DISCUSSION

Phytochemical investigation of plant products were essential to detect bioactive principles which are new sources of industrially and therapeutically valuable compounds that may lead to the discovery of new drugs. In pharmaceutical companies, crude plant extracts and medicines are manufactured based on the principles of natural compounds, may lead to large scale exposure of humans on natural products. Presence or absence of essential bioactive compounds in plant extracts were identified by color reactions with specific chemicals, this procedure is simple for preliminary pre-requisite before going to phytochemical investigation. In the present research work, phytochemicals were screened with the ethanol, acetone and aqueous extracts of the *lawsonia inermis* leaves and the results are shown in Table 1.

**Table 1: Preliminary phytochemical investigation of *lawsonia inermis* leaves.**

Test	Ethanol	Acetone	Aqueous
Alkaloids	+	-	+
Steroids	+	+	+
Tannin	+	+	+
Phlobatanins	+	+	-
Saponins	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Aminoacids	+	-	+
Glycosides	+	+	+
Reducing sugars	+	-	+

+indicates present, -indicates absent.

The present research work recorded and highlighted that ethanolic extracts of *lawsonia inermis* leaves had the highest number of phytochemicals compared with other solvent extracts. Hence, ethanolic extracts of *lawsonia inermis* leaves hold the great potential to treat various human diseases and has profound medical applicability. Due to presence of secondary metabolites, the plant leaf extract exhibits anthelmintic activity to bind free proteins

in the gastrointestinal tract of host animal and glycoprotein on the cuticle of the parasite. The results of anthelmintic activity on earthworms *pheretima posthuma* was shown in Table-2. Different concentrations (10, 25 and 50mg/ml) prepared for evaluation of paralysis and death of earthworms and it was compared with the same concentration for standard drug.

**Table 2: Anthelmintic activity of Plant Extracts.**

Groups	Dose in concentration (mg/ml)	Time of paralysis (min)	Time of death (min)
Acetone extract	10	19.45±0.14	33.5 ± 0.41
	25	15.20± 0.52	25.18±0.14
	50	12.38 ± 0.27	17.38 ± 0.26
Ethanolic extract	10	17.17 ± 0.32	31.27 ± 0.43
	25	13.31±0.41	20.63 ± 0.42
	50	10.63 ± 0.33	12.43 ± 0.36
Aqueous extract	10	18.28±0.33	36.63 ± 0.42
	25	14.82± 0.31	27.09± 0.15
	50	11.51 ± 0.27	14.38 ± 0.42

Standard Drug	10	8.44 ± 0.41	12.26 ± 0.52
	25	6.12 ± 0.54	9.36 ± 0.43
	50	2.39 ± 0.19	4.43 ± 0.34

## CONCLUSION

The present research work concluded that *lawsonia inermis* leaves contain potentially health-protective phytochemical compounds with a potent source of natural antioxidants and antibacterial activities that may be clinically promising. The plant *lawsonia inermis* leaves have been used in the treatment of different ailments, the medicinal roles of this plant could be related to identify bioactive compounds. The present results will form the basis for collecting of new plant species for further investigation in the potential discovery of new bioactive compounds. Further studies are required for in-vitro activity, to establish effectiveness and pharmacological rationale for the use of leave extracts as anthelmintic drug. The present research concluded that the plant *lawsonia inermis* leaves extract has significant anthelmintic activity on ethanolic extract.

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