



## In Vitro Evaluation of Anthelmintic Activity of Ethanolic Extract of *Ipomoea Aquatica* Forsk. In Indian Adult Earthworms

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### Abstract

**Introduction:** Helminthiasis is one of the most prevalent parasitic infestations globally accounting for major threat to public health. Conventionally only few anthelmintic drugs are available for the control of these nematodes. However, emerging resistance and toxicities to these currently available drugs is a major concern and discovery of newer anthelmintics with a novel mode of action is the need of the hour.

**Aims and Objectives:** The present study is aimed to evaluate the anthelmintic activity of ethanolic extract of *Ipomoea aquatica* Forsk. on Indian earthworms (*Pheretimaposthuma*).

**Materials and Methods:** The earthworms were divided into 4 groups with 6 worms in each group. The anthelmintic activity of EEIA at two different concentrations (25 mg/ml & 50 mg/ml) was evaluated by assessing the time of paralysis and time of death of the worms. Albendazole was used as standard & 2% gum acacia as control.

**Results:** Albendazole at 25 mg/ml showed the highest anthelmintic activity and had significant difference ( $p < 0.001$ ) with EEIA at both 25 mg/ml & 50 mg/ml. Both doses of the test drug showed anthelmintic activity but the extract at either dose was found to be less effective than the standard drug.

**Conclusion:** Further studies with higher doses of the extract should be done to evaluate the anthelmintic activity in a dose-dependent manner.

**Keywords:** Albendazole, Helminthiasis, *Ipomoea aquatica* Forsk, *Pheretimaposthuma*.

### Introduction

Helminthiasis is currently one of the most prevalent parasitic infestations globally affecting around 1/3<sup>rd</sup> of the world's population. As per WHO, around 1.5 billion people worldwide are infested with soil-transmitted helminths (STH)<sup>[1]</sup>. India has the highest burden of STH among all the countries contributing to one-fourth of total global

cases which is prevalent among children of age group 1-14yrs<sup>[2]</sup>. Overall prevalence of STH in India ranges from 13% to 66%<sup>[3]</sup>. Intestinal worms can cause health hazards like undernourishment, anaemia, eosinophilia, pneumonia and in extreme cases even death<sup>[4]</sup>.

Synthetic anthelmintics have been used extensively all over the world for decades to

minimize the losses caused by helminth infections<sup>[5]</sup>. The currently available anthelmintic drugs exert their action by incapacitating the parasite by causing damage and paralysis of the worm so that the immune system can eliminate it, or by altering its metabolic processes<sup>[6,7]</sup>. But the emergence of resistance to the currently available AHDs is a major concern and newer anthelmintics with a novel mode of action has become necessary<sup>[8]</sup>.

Phytochemical control of gastrointestinal nematodes presents an important area of research, given its historical and traditional background, and incidental application in most communities around the world. Herbal drugs in recent years have become popular and also gained importance due to their safety, efficacy and cost effectiveness in treating various diseases. *Ipomoea aquatica* Forsk. known as 'Kalmisag' in Hindi, 'Kalmisak' in Bengali, 'Kolamni' in Manipuri, 'Water spinach' in English is a medicinal plant that has been in use for the treatment of a wide range of diseases. The herb is used for the treatment of liver problems, diabetes, abscess, mental illness, intestinal problems. It is also used in epistaxis, high blood pressure, helminthiasis, hyperlipidemia, infectious diseases etc. Juice of this plant is used as purgative, antiemetic and as antidote in opium and arsenic poisoning. It also has role in the inhibition of prostaglandin synthesis<sup>[9]</sup>. *Ipomoea aquatica* Forsk. contains phytochemical constituents like flavonoids, alkaloids, saponin, carbohydrates, glycosides, steroids, proteins and amino acids and tannins<sup>[10]</sup>. As such, considering its use in gastro-intestinal ailments by locals, the present study was aimed at the evaluation of *in vitro* anthelmintic effect of ethanolic extract of leaves of *Ipomoea aquatica* Forsk. (EEIA) on Indian earthworms (*Pheretima posthuma*).

## Materials and Methods

### Collection of plant Materials

The present study was conducted in the Department of Pharmacology. The plant *Ipomoea*

*aquatica* Forsk. was collected from local market was authenticated by the Department of Botany, D.M. College having Voucher no. 001351.

### Preparation of plant extract

The leaves of the plant *Ipomoea aquatica* Forsk. were shade dried and powdered using a mixer grinder. Powdered dry plant material (50 gm) was extracted with 100 ml ethanol for 24 hrs and thus ethanolic extract was obtained using soxhlet apparatus manufactured by Jain Scientific Glass Works, Ambala Cantt, Haryana, India. The extract was filtered and concentrated under vacuum-sounding apparatus for 30 min and then stored at 4°C<sup>[11]</sup>. The percentage yield was 18% and the extract thus obtained was used for the anthelmintic study.

### Worm collection and authentication

The study was done on adult Indian earthworm *Pheretima posthuma* because of its similar anatomical and physiological features to *Ascaris lumbricoides*<sup>[12,13]</sup>. The earthworms (5-8 cm in length) were collected from moist soil and authenticated by the Department of Life Sciences, Manipur University. They were cleaned with normal saline (NS 0.9%) to remove soil and faecal matter.

### Phytochemical Analysis

The preliminary phytochemical analysis of the plant extract was carried out using a standard procedure to identify various constituents<sup>[14]</sup>.

### Experimental Design

The earthworms were divided into 4 groups with six worms in each group. Standard and test drugs were dissolved in 2 % gum acacia and 10 ml of the desired formulation were poured in separate petri dishes and were kept under room temperature. The earthworms were placed in the petri dishes containing the extract solutions or the standard drug as mentioned in Table 1.

**Table 1:** Allotment of earthworms in different groups and their treatment

Group	Treatment
1 (Control)	2% gum acacia
2 (Standard)	Albendazole suspension at a dose of 25mg/ml
3 (Test A)	EEIA at a dose of 25mg/ml
4 (Test B)	EEIA at a dose of 50mg/ml

### Evaluation of anthelmintic activity

The living or viable worms were kept under close observation. External stimuli was frequently applied to each worm which stimulated and induced movement in live earthworms. Time taken for paralysis of the worms were noted when no spontaneous movement could be observed except when shaken vigorously. Time taken for death of worms were recorded when worms did not move even after vigorous shaking or when dipped in warm water (50°C) which was followed by gradual fading of their body color<sup>[15]</sup>.

### Statistical Analysis

Data obtained was analysed using One way ANOVA followed by Bonferroni test post hoc. Results were expressed in Mean  $\pm$  SEM.

### Results

The ethanolic extract of leaves of *Ipomoea aquatica* Forsk. (EEIA) had significant anthelmintic activity when compared with control ( $p < 0.001$ ). The standard drug albendazole at 25 mg/ml showed the best activity for time of paralysis and death ( $16.42 \pm 2.51$  and  $21.13 \pm 2.32$  minutes respectively), while EEIA (25 mg/ml) showed  $42.01 \pm 3.09$  and  $46.19 \pm 2.49$  minutes respectively and EEIA (50 mg/ml) showed  $30.15 \pm 2.56$  and  $35.09 \pm 3.27$  minutes respectively for time of paralysis and death (Table 2). Moreover, higher dose of EEIA (50 mg/ml) showed significant ( $p < 0.001$ ) increased anthelmintic activity by the reduced time of paralysis and death. However, the extract at either dose was found to be less effective than the standard drug.

**Table 2:** In vitro anthelmintic activity of EEIA on Indian earthworms

Group	Drugs	Time of paralysis (minutes)	Time of death (in minutes)
1	Control (2% gum acacia)	–	–
2	Standard (Albendazole 25mg/ml)	$16.42 \pm 2.51$ #	$21.13 \pm 2.32$ #
3	Test A (EEIA 25mg/ml)	$42.01 \pm 3.09$ *	$46.19 \pm 2.49$ *
4	Test B (EEIA 50 mg/ml)	$30.15 \pm 2.56$ *#	$35.09 \pm 3.27$ *#

n=6 in each group, values are Mean  $\pm$  SEM. Symbols \*, # were used to compare with groups 2 and 3 respectively.  $p < 0.001$

### Discussion

The occurrence of resistance to the currently available anthelmintic drugs and their associated toxicities have led to a renewal of interest in developing plant-derived medicines. Secondary metabolites produced by a large number of plants of may serve as future reservoir for novel drugs and therapeutic agents. Evaluation of anthelmintic activity of new plant compounds by *in vitro* methods using free living stages of parasitic nematodes have become popular<sup>[16]</sup>. *In vitro* techniques are cost effective, simple and have rapid turnover compared to *in vivo* methods<sup>[17]</sup>.

The preliminary phytochemical analysis shows presence of tannins, alkaloids, flavonoids, saponin. Tannins can cause uncoupling of oxidative phosphorylation and thus interfere with energy generation in helminths. It can also bind to the free proteins in the GIT of host or glycoprotein on the cuticle of the parasite and causes death of the worms<sup>[18]</sup>. Phytochemical analysis also reveals presence of saponin which can stabilize the membrane permeabilizing and also can forms pores. Permeability of the cell membrane of the parasites is thus affected which forms vacuoles, resulting in disintegration of monogenea teguments<sup>[19]</sup>. Presence of alkaloids in the extract can block intake of acetylcholine from the host which expels out the worms by peristaltic movement of intestine<sup>[20]</sup>.

Conventional anthelmintic drug albendazole is safe and effective for parasitic infections and an established standard drug in anthelmintic studies. It is thought to inhibit microtubule polymerization by binding to  $\beta$ -tubulin. The selective toxicity of this agent against helminths results from higher affinity for parasite  $\beta$ -tubulin than for the same target in higher eukaryotes<sup>[6]</sup>. The least time taken for paralysis and death of worms by albendazole correlates with previous studies<sup>[15]</sup>. The extract is found to be more effective at 50 mg/ml but the extract at either dose was found to be less effective than the standard drug.

## Conclusion

The discovery of a potent remedy from plant origin will be a great advancement in the treatment of helminthic infections. Use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. The current study evidenced that the ethanolic extracts of *Ipomoea aquatica* has a promising in vitro anthelmintic activity. However further studies with higher doses of the plant are required to evaluate the dose dependent anthelmintic activity and also to determine the active principle responsible for exact mechanism of anthelmintic activity.

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