

## INVESTIGATION OF IN VITRO ANTHELMINTIC ACTIVITY OF *Clerodendron Inerme*

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### ABSTRACT

The aqueous extract of *Clerodendron inerme* leaves was investigated for anthelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (10-50 mg/ml) of plant extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintic activity at the concentration of 30 mg/ml. The result shows that aqueous extract possesses vermifugal activity and found to be effective as an anthelmintic. Therefore, the anthelmintic activity of the aqueous extract of *Clerodendron inerme* has been reported for the first time.

**Keywords:** Anthelmintic, *Clerodendron inerme*, the village Bahirkunja, vermifugal.

### Introduction

Infections with helminth are among the most widespread infections in humans and other domestic animals affecting a large number of world population. The majority of these infections due to worms are generally restricted mainly to the tropical regions and the occurrence is accelerated due to unhygienic lifestyle and poverty also resulting in the development of symptoms like anaemia, eosinophilia and pneumonia<sup>1</sup>. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas<sup>2</sup>. The gastrointestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases<sup>3</sup>. Therefore, the sensitive anthelmintics from plant resources must be developed.

*Clerodendron inerme*, green stem shrub grows to the

light. Height is about 2-3 meters, width varies from 15 to 25 cm, light requirement is Low to High, Temperature is 20°C to 30°C, Hardness tolerance is very soft to very hard, pH tolerance is 4 to 7, growth is moderate. Common name is Bonghama in Bengali, synonym is *Clerodendrum inerme*, English name is Glory Bower genus, Family – verbenaceae, Genus – clerodendron, Species - inerme. *Clerodendron inerme* is a branched shrub, 2 to 3 meters high, the stems woody and smooth. Leaves are simple and opposite. Flowers are rotationally symmetric, salverform, white--symmetry, sex, arrangement of male and female flowers if unisexual.

Geographical distribution: *Clerodendron inerme* is mainly found in India, Nepal and Bangladesh. It is mainly found in the terrestrial and tropical regions.

Cultural usage: Leaves used in the treatment of intestinal worms, fever, cough and diarrhoea, also used

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in conjunction with other plants leaves for ghost diseases.

*Clerodendron inerme*, a tropical plant, widely distributed in villages of West Bengal and reported as medicinally important plant. We have chosen this plant for the detail pharmacological studies to prove whether this plant can be used as an enormous source for future medicinal development.

On the side of the river Hoogly in West Bengal there are many villages situated and Bahirkunja is one of them. Bahirkunja is full of medicinal plants and this area is far away from city kolkata. Therefore, this area is lack of the light of modern medicine. Therefore, the poor people are dependent on several plants or plant-based preparations for the treatment of various ailments in their traditional system of medicine. During our course of studies on ethnomedicine of this area, the plant being used as anthelmintic is leaves of *Clerodendron inerme*. This plant has a wide reputation among natives of being curative for intestinal-worm infections. This plant is being used by the villagers of Bahirkunja as an anthelmintic in the form of extract, prepared by soaking powdered material in water for 10-12 hours. This extract is taken orally once a day for three days to treat intestinal-worm infections. We have also watched that the poor villagers of Bahirkunja are using the raw juice of the leaves of *Clerodendron inerme* as anthelmintic for domestic animals such as cow, dog, goat etc. Based on this, an attempt has been made to evaluate the anthelmintic potential of *Clerodendron inerme*.

## **Materials and methods**

### **Plant Materials**

The plant *Clerodendron inerme* (Family: verbenaceae) was collected from the village (Bahirkunja and Amtala) south 24 paraganas district, West Bengal, India. The plant material was taxonomically identified by the

taxonomists of Botanical Survey of India, Kolkata. A voucher specimen has been preserved in our laboratory for future reference. The plant material was dried in shade, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.

### **Preparation of extract**

#### **Aqueous extract (Maceration method)**

The plant leaves were dried in shade, pulverized and then powdered material of *Clerodendron inerme* leaves (500gm) were kept for maceration with 1000 ml of double distilled water for 24 hours. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be about 4%.

### **Experimental Animals**

Adult earthworms (*Pheretima posthuma*), Roundworm (*Ascaridia galli*) and Tapeworms (*Raillietina spiralis*) were used to evaluate anthelmintic activity *in vitro*. Earthworms were collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter. These intestines were then dissected and worms were collected and kept in normal saline solution. The average size of round worm was 5-7 cm and average size of tapeworm was 6-8 cm. Earthworm and helminths were identified in Dept. of Zoology, Vivekananda College, Thakurpukur, Kolkata.

### **Drugs & Chemicals**

Piperazine citrate (Glaxo Smithkline) was used as standard anthelmintic during the experimental protocol.

**Anthelmintic activity**

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al <sup>4</sup>. The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) as it is having anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation of anthelmintic activity <sup>5,6,7</sup>. Use of *Ascaridia galli* and *Raillietina* species as a suitable model for screening of anthelmintic drug was advocated earlier <sup>8,9</sup>. Test samples of the extract was prepared at the concentrations, 10, 20,30,40 and 50 mg/ml in distilled water and six worms i.e. *Pheretima posthuma*, *Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg /ml) was used as reference standard and double distilled water as control <sup>10,11,12,13</sup>. This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when

dipped in warm water (50<sup>0</sup> C). All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

**RESULTS AND DISCUSSION**

From our observations, higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for all types of worms. Aqueous extract of *Clerodendron inerme* exhibited anthelmintic activity in dose-dependent manner showing maximum efficacy at 30 mg/ ml concentration for all three types of worms. Our plant extract exhibited more potent activity at lowest concentration (10 mg/ml) against (roundworm) *Ascaridia galli*. Anthelmintic activity of the extract was compared with the standard drug Piperazine citrate (Table.1). From the above results, we can conclude that *Clerodendron inerme* which is used traditionally to treat intestinal worm infections, exhibited significant anthelmintic activity. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

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**Table 1:** Anthelmintic activity of *Clerodendron inerme* ( verbenaceae) aqueous extract

Groups	Conc. (mg/ml)	<i>Pheretima posthuma</i> (Earthworm)		<i>Ascaridia galli</i> (Roundworm)		<i>Raillietina spiralis</i> (Tapeworm)		
		Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	
01	Control (Water Only)	-----	-----	-----	-----	-----	-----	
02	<i>Clerodendron inerme</i>	10	30±0.41	50±0.22	27±0.35	55±0.42	32±1.50	60±1.50
		20	25±0.12	45±0.27	22±0.75	50±0.31	24±0.40	45±0.52
		30	18±0.50	30±0.63	15±0.18	39±0.50	20±1.62	40±1.10
		40	17±0.32	30±0.11	13±0.85	38±1.20	19±0.50	40±0.50
		50	17±0.50	28±0.25	11±0.37	36±1.12	18±0.75	39±1.20
03	Piperazine Citrate (standard)	10	22±1.10	60±0.75	12±1.50	38±1.10	24±0.50	52±0.10

Each value represents mean ± SEM (N=6).

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