



A STUDY ON CYTOTOXIC AND ANTHELMINTIC ACTIVITIES OF CRUDE EXTRACTS OF LEAVES OF *CLERODENDRUM VISCOSUM*

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ABSTRACT

This study describes the biological investigations of *Clerodendrum viscosum* – a plant belonging to the family Verbenaceae. The leaf of *Clerodendrum viscosum* was extracted with methanol and water. The crude extract of methanol of *Clerodendrum viscosum* was screened for cytotoxic activity using brine shrimp lethality bioassay. A reputed cytotoxic agent vincristine sulphate was used as a positive control. From the result of the brine shrimp lethality bioassay it can be well predicted that methanol extract possess cytotoxic principles (with LC₅₀ 3.696 µg/ml) comparison with positive control vincristine sulphate (with LC₅₀ 0.773 µg/ml). Both methanolic and aqueous extracts from the leaves of *Clerodendrum viscosum* were investigated for their anthelmintic activity against *Pheretima posthuma* and five concentrations (10, 20, 30, 40 and 50 mg/ml) of each extracts were studied in activity, which involved the determination of time of paralysis and time of death of the worm. Both the extracts exhibited significant anthelmintic activity at highest concentration of 50 mg/ml. Albendazole in 20mg/ml concentration extract was used as standard reference and saline solution as control. All the extracts showed significant anthelmintic activity in dose dependent manner.

Key words: Biological investigation, Lethality, Cytotoxic Activity, LC₅₀, Anthelmintic Activity, Positive control, Methanolic Extract.

INTRODUCTION

Medicinal properties of the plants are due to the phytochemical present in them and whose therapeutic application can be established in light of modern medicine. The World Health Organization (WHO) estimates that 4 billion people, 80 percent of world population presently use herbal medicine for primary health care.¹

The plant Kingdom still holds many species of plants containing active constituents of medicinal value which have yet to be screened for their pharmacological value in addition to the already exploited plants. New plants drugs usually find their way to modern medicine by use of modern isolation techniques and pharmacological screening methods (Wermuth 2003).

Clerodendrum viscosum (Verbenaceae: Bhat in Bengali, Bhania in Oriya, Parugilai, Perugilai, Vattakanni in Tamil, Vatta-perivalam, Paragam in Malayalam, Glory tree in English) is a shrub of 0.9 to 2.4 m height and is found abundantly in Bangladesh, India, Pakistan, Ceylon, Burma, and Malaysia. It is a terrestrial shrub having square, blackish stem and simple, opposite, decussate, petiole, exstipulate, coriaceous, hairy leaves with a disagreeable odor.

The plant is common throughout the plains of Bangladesh. Use of leaves and roots are commonly found in traditional medicines. The leaf and root have been reported to possess a number of therapeutic activities to manage diseases state like convulsion, diabetes, gravel, malaria, sore, spasm and scorpion sting. Fresh leaf juice widely used as antidandruff, antipyretic, laxative and vermifuge. The leaves and roots are externally used for tumors and in certain other skin diseases as paste. The leaves are also used in snake bite and scorpion sting in ayurveda but contradictory reports have been published about the anti venom property of the leaves. In Thai medicine the leaves and root are known to be diuretic; and used for treatment of intestinal infections and kidney dysfunction.²

The present study has been designed to evaluate cytotoxic activity of the ethanol extract and anthelmintic activity of the both ethanol and aqueous extract of the leaves of *Clerodendrum viscosum* which can help in authenticating

the sample in future study. Traditionally, the plant is used as an aphrodisiac, antipyretic, and anthelmintic. Hence present study was undertaken to prove cytotoxic activity of extracts of *Clerodendrum viscosum* against brine shrimp and anthelmintic activity against selected earthworms.

MATERIALS AND METHODS

Collection and identification of the plant:

Plant sample (leaf) of *Clerodendrum viscosum* was collected from Companygong, Noakhali in April 2011. Then the plant is identified by the Bangladesh National Herbarium and collects an accession number- DACB: 35979

Extraction of plant constituents (cold extraction)

The leaves of the plant were collected in fresh condition. It was sun-dried to make it suitable for grinding purpose. The coarse powder was then stored in air-tight container with marking for identification and kept in cool, dark and dry place for future use. The leaves were washed thoroughly to remove adhered material. The powdered 350 gm powders were soaked in 1.65 liters methanol in September 22, 2011 in desiccator, through daily 2 hours shaking and stirring for 7 days. The extract was then filtered through filter-cloth. The filtrate was kept to dry in fresh and clean air to afford a mass of biological investigation.

Cytotoxic activity screening

Drugs and chemicals

1. DMSO (dimethyl sulfoxide)
2. Vincristine sulphate

Cytotoxic activity

The cytotoxicity assay was performed on brine shrimp nauplii as reported in Meyer et al. 1982.^{3,4,5,6} Brine shrimp nauplii were obtained by hatching brine shrimp eggs in artificial sea-water (38gm sodium chloride dissolved in 1000 ml distilled water) for 48 hours. Dissolution of extract was performed in artificial sea-water by using DMSO (dimethyl sulfoxide). Each 5 ml solution of nine different concentrations (1.5625, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml) of the extract was taken in nine different beakers containing nine living brine shrimp nauplii. The assay is performed using three replicates and observed for mortality

after 24 hours. These data are processed in a simple program for Probit Analysis to estimate LC₅₀ values with 95% confidence intervals for statistically significant comparisons of potencies. Vincristine sulphate was used as positive control. A negative control group was also prepared containing sea water and 100µl DMSO.

Anthelmintic activity screening

Experimental model

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity in vitro. Earthworms were collected near the swampy water along Noakhali Science and Technology University road, Sonapur, Noakhali. All the worms were washed with normal saline to remove all fecal matters. The earth worms 5–7 cm in length and 0.3–0.4 cm in width weighing 0.8–3.04 g were used for all experiment protocols.

Drugs and chemicals

Albendazole

Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al. 2001.⁷ with minor modifications. The assay was performed in vitro using adult earthworm (*Pheretima*

posthuma) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity (Gboladeet al. 2008).^{8,9,10,11}

Preparation steps:

100, 200, 300, 400, 500 mg of extract was weighed for leaves extract. Then they were dissolved in 10 ml of distilled water in volumetric flask. Thus the concentrations of the solutions are respectively 10, 20, 30, 40 and 50 mg/ml. 200 mg of albendazole was dissolved in 10 ml of distilled water. Now the concentration is 20mg/ml. Earthworms were divided into twelve groups each containing three worms in petri dish. In five groups Leaves extract was applied. And one is for reference and one is for negative control

Observation steps:

Observations were made for determining the time taken for paralysis and death of the worm. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lose their motility followed with fading away of their body color.

Table 1: Results of the test sample of methanolic extract of *Clerodendrum viscosum*

Sample	LC ₅₀ (µg/ml)	Regression equation	R ²
Vincristine sulfate (positive control)	0.773	y = 32.66x + 53.66	0.935
Methanolic extract	3.696	y = 32.11x + 31.77	0.824

Table 2: Effect of methanolic extract and vincristine sulfate on shrimp nauplii

Methanolic Extract				Vincristine sulfate			
Conc. (µg/ml)	log C	% Mortality	LC ₅₀ (µg/ml)	Conc. (µg/ml)	Log C	% mortality	LC ₅₀ (µg/ml)
400	2.60206	100		40	1.60206	100	
200	2.30103	100		20	1.30103	90	
100	2	100		10	1	90	
50	1.69897	100	3.696	5	0.69897	80	
25	1.39794	80		2.5	0.39794	70	0.773
12.5	1.09691	80		1.25	0.09691	70	
6.25	0.79588	70		0.625	-0.20412	50	
3.125	0.49485	40		0.3125	-0.50515	30	
1.5625	0.19382	20		0.15625	-0.80618	20	

Table 3: Anthelmintic activity of extracts of *Clerodendrum viscosum*

Extracts	Concentration mg/ml	<i>Pheretima posthuma</i>	
		P	D
ME	10	50.03 ± 0.2	130.05 ± 0.3
	20	32 ± 0.12	94 ± 0.96
	30	24.07 ± 0.5	70.23 ± 0.11
	40	14.3 ± 0.67	46 ± 0.87
	50	8.8 ± 0.98	30 ± 0.30
WE	10	144.75 ± 1.7	318.15 ± 0.3
	20	110 ± 0.56	265 ± 0.07
	30	86.75 ± 0.4	220.9 ± 0.5
	40	70 ± 0.12	180 ± 0.54
	50	62.25 ± 0.81	120.34 ± 0.02
Albendazole	20	17.67 ± 0.54	48 ± 0.47
Control	0	----	----

Where ME: Methanolic extract, WE: Water extract P: Time taken for Paralysis (min), D: Time taken for Death of worms (min)

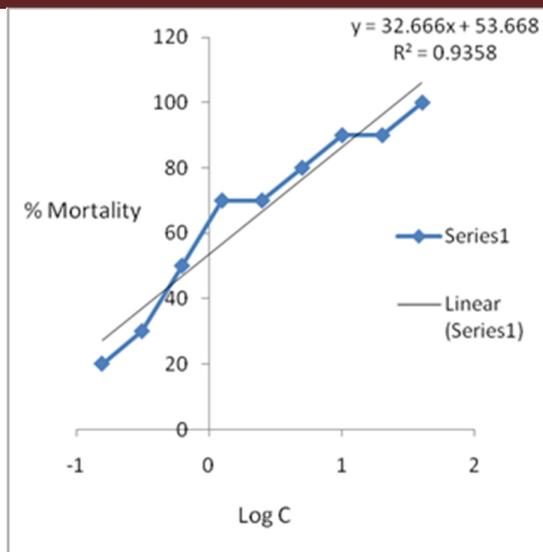


Figure 1 (a) Effect of vincristine sulfate on shrimp nauplii

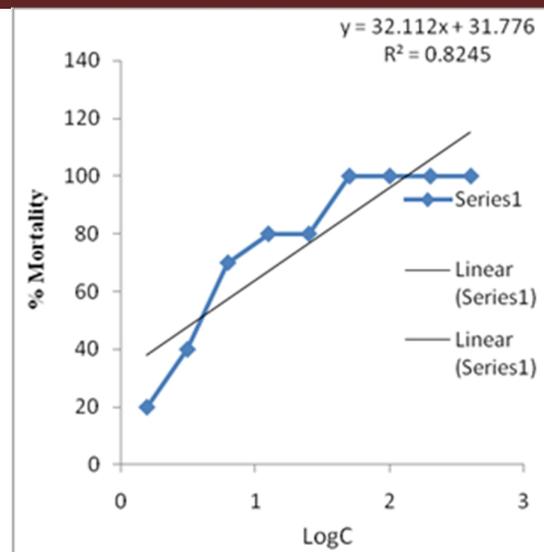


Figure 1(b) Effect of methanolic extract on shrimp nauplii

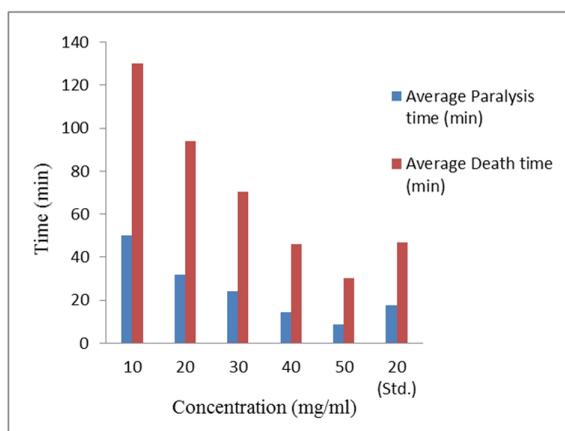


Figure 2: Concentration vs. Average paralysis and death time of methanolic extract

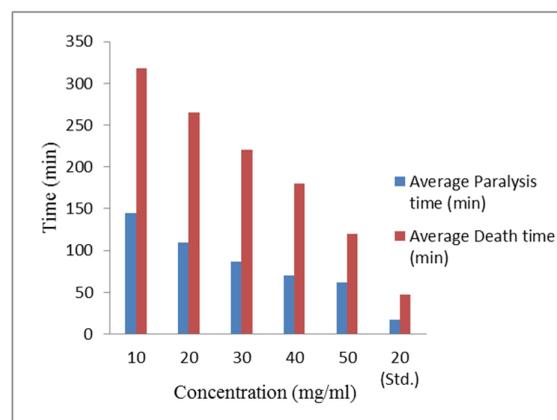


Figure 3: Concentration vs. Average paralysis and death time of water extract

RESULTS AND DISCUSSION

Cytotoxic activity

Cytotoxic activity of *Clerodendrum viscosum* leaves extract was determined by brine shrimp lethality assay. Percentage mortality of brine shrimp at nine different concentrations of crude extract was shown lethality in a dose dependent manner. Following the procedure of Meyer (Meyer et al., 1982) the lethality of the methanolic extract was determined and the summary of the result is expressed in table 1 and table 2. The LC_{50} value of the methanolic extract is $3.696\mu\text{g/ml}$. And the vincristine sulfate showed LC_{50} at concentration of $0.773\mu\text{g/ml}$ (Table 1)

From the results of the brine shrimp lethality bioassays it can be well predicted that the methanolic extract possess cytotoxic principles. Comparison with positive control vincristine signifies that cytotoxicity exhibited by the methanolic extract might have moderate antitumor activity. In Figure 1(a) shown effect of vincristine sulfate on shrimp nauplii, also Figure 1(b) shown that effect of methanolic extract on shrimp nauplii. However this can not be confirmed without further higher and specific tests.

Anthelmintic activity

Both the extract showed significant anthelmintic activity on selected worms. Methanol extract found to be more active as compared to water extracts. The methanol extracts

demonstrated paralysis as well as death of worms in a less time as compared to water extraction in case of *Pheretima posthuma* (Table 3). Figure 2 shown that Concentration vs. Average paralysis and death time of methanolic extract and figure 3 also shown Concentration vs. Average paralysis and death time of water extract. Although water extracts also shown significant activity. Phytochemical analysis of the crude extracts revealed presence of saponin, steroids, alkaloids, triterpenoids and bitter principles as are the chemical constituents. Triterpenoids and bitter principals are known to have anthelmintic activity.

The pharmacological investigation for cytotoxic properties of crude extract (methanolic extract) of *Clerodendrum viscosum* by using brine shrimp lethality bioassay showed a moderate cytotoxicity in comparison with positive control vincristine sulphate. However this can not be confirmed without further higher and specific tests. In this study, the use of leaves of *Clerodendrum viscosum* as an anthelmintic have been confirmed as the leaves extracts (both methanol and aqueous) displayed activity against the worms used in the study. Further studies involving in vivo models of anthelmintic activity to establish the mechanisms of action are required.

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