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Evaluation of Cytotoxic and Anthelmintic Activity of Plant Roots of *Clerodendrum viscosum* (Family: Verbenaceae)

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ABSTRACT

In the present study, *in-vitro* cytotoxic and anthelmintic activity of root extract of *C. viscosum* was evaluated. The Brine shrimp lethality bioassay method was used to determine the cytotoxic activity and vincristine sulphate was used as positive control. The plant extracts were also evaluated for *in-vitro* anthelmintic activity against Bangladeshi earthworms *Pheretima posthuma* and the time taken for worm paralysis and death was determined. One way analysis of variance (ANOVA) was used to determine the level of significance. The methanolic crude extract of plant root exhibited mild to moderate cytotoxicity with LC₅₀ value of 10.235±1.061 µg/ml (95% CI: 7.60-12.87) as compared with vincristine sulphate with LC₅₀ value of 0.841±0.025 µg/ml (95% CI: 0.78-0.90) and the difference was statistically significant (p<0.05). In the screening of anthelmintic activity, methanolic crude extract as well as aqueous fraction (water) of plant root revealed statistically significant anthelmintic activity on adult Bangladeshi earth worm *Pheretima posthuma* in comparison to the reference drug (p<0.05). The paralyzed time of methanolic crude extract, aqueous fraction and standard Albendazole were 13.23±0.49, 78.24±0.17 and 17.67±0.54 minutes at a concentration of 50 mg/ml. The death times of previous three samples were 45.10±1.79, 177.37±0.62 and 48±0.47 minutes respectively at the same concentration. In conclusion, our study suggests that the crude extracts of *C. viscosum* roots possess some action of cytotoxic and anthelmintic activities.

Keywords: *Clerodendrum viscosum*, cytotoxic activity, Anthelmintic activity, Lethal Concentration, *Pheretima posthuma*.

1. Introduction

The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects^[1]. Secondary metabolites of plants play an important role in health care for about 80% of the world's population^[2]. Approximately, half of the world's 25 best-selling pharmaceutical agents are derived from natural products^[3]. Thus, emphasis is now given on the standardization of herbal medication by screening of biological activities of medicinal plants and isolating active principles from them^[4].

The genus *Clerodendrum* L. (Family: Lamiaceae) is very widely distributed in tropical and subtropical regions of the world as a medicinal plants. More than five hundred species of the genus are identified till now, which includes small trees, shrubs and herbs. The plant *Clerodendrum viscosum* is an indigenous medicinal plant widely distributed in various parts of India, Ceylon, Malaya and Bangladesh^[5]. In Bangladesh, among the shrubs, the highest density (53.57 plants/100 m²) and frequency (35.71%) were found in *C. viscosum*^[6]. Local names are Bhand, Hill Glory Bower, Bag flower and Bleeding-heart. Other species of *Clerodendrum* including *C. phlomidis*, *C. inerme*, *C. colebrookianum* and *C. trichotomum* possess significant biological activity such as antioxidant, anthelmintic, cytotoxic and antitumor activity^[7, 8]. Recently a study also identified significant cytotoxic and anthelmintic activity of leaves extract of *C. viscosum*^[9].

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But to the best of our knowledge, previously no study was conducted to determine the cytotoxic and anthelmintic activity of root extract of *C. viscosum*. Due to the lacking of information, we intended to evaluate the cytotoxic and anthelmintic activity of plant roots of *C. viscosum* (Family: Verbenaceae).

2. Materials and Method

2.1. Collection, Authentication and Processing of Plant Materials

The fresh roots of *C. viscosum* were collected in the months of April-May, 2012, from the road side of Companygonj, Noakhali, Bangladesh, and authenticated by the authority of the Bangladesh National Herbarium, Mirpur, and Dhaka. A voucher specimen was submitted at Bangladesh National Herbarium for future reference. After identification of the plant by the Bangladesh National Herbarium, an accession number (DACB: 35979) was collected. The root (after cutting into small pieces) was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature (40 °C) for better grinding. The dried samples were then ground in coarse powder using high capacity grinding machine. The coarse powder was stored in air-tight container with marking for identification and kept in cool, dark and dry place for future use.

2.2. Preparation of crude extract

2.2.1. Methanolic extract

The powdered material (400 g) was taken in separate clean, round bottomed flask (2.5 liters) and soaked in 1.5 liters of methanol. The container with its content was sealed by cotton plug and aluminium foil and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton plug followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 40 °C with a rotary evaporator. The concentrated extract was then air dried to solid residue. The weight of the methanol soluble extract was obtained 2.5 g.

2.2.2. Water extract

For Water extraction fresh roots are needed for grind. So, after collection it should grind with water as early as possible. Aqueous extract was obtained by maceration for 24 hours. The roots were washed thoroughly to remove adhered material. Then 20 g fresh roots were grind thoroughly in 500 ml distilled water. The material was filtered through filter cloth and filtrate was collected. Finally the liquid was dried in air at ambient temperature (25 °C) to obtained water extract.

2.2.3. Evaluation of Cytotoxicity

There are sufficient experimental evidences reporting the use of brine shrimp for environmental studies^[10, 11], screening for natural toxins^[12] and as a general screening for bioactive substances in plant extracts^[13]. In the present study, we used this animal to evaluate the cytotoxic activity of *C. viscosum*. For the study purpose, *Artemia salina* as a test object and a developed protocol was used in Brine shrimp lethality bioassay to monitor cytotoxicity of *C. viscosum*^[14]. Brine shrimp eggs were hatched in simulated sea water to get nauplii. Sample solutions were prepared by dissolving the test materials in pre-calculated amount of dimethyl sulfoxide (DMSO). Ten nauplii were taken in vials containing 5 ml of simulated sea water. The samples of different concentrations were added to the pre-marked vials with a micropipette. The assay was

performed using three replicates. Survivors were counted after 24 hours. 38 g sea salt (pure NaCl) was weighed, dissolved in one liter of distilled water and filtered off to get clear solution.

A. salina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of a Pasteur pipette 10 live shrimps were added to each of the vials containing 5 ml of seawater. Clean test tubes were taken. These test tubes were used for ten different concentrations (one test tube for each concentration) of test samples and ten test tubes were taken for standard drug Vincristine sulphate for ten concentrations of it and another one test tube for control test. Then 100 µl of solution was taken in test tube each containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg/ml and others are 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 µg/ml. Vincristine sulphate (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.0781 and 0.039 µg/ml) was used as positive control. A negative control group was also prepared containing sea water and 100 µl DMSO. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

2.2.4. Evaluation of anthelmintic activity

In vitro anthelmintic activity can be screened using several worm samples like *Ascaridia galli*, *Ascaris lumbricoides* and *Pheretima posthuma*. But *P. posthuma* was most frequently used as test worm in anthelmintic activity determination, because it possesses anatomical and physiological similarity with the intestinal roundworm parasite of human^[15]. The anthelmintic activity was performed according to the method of Ghosh *et al.*^[16] on adult Bangladeshi earthworm *Pheretima posthuma*. The earthworms (*P. posthuma*) were collected from moist soil and washed with normal saline to remove all fecal 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. Various concentrations of each extract (Methanol and Water) were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference and saline solution as control.

Various weight of roots extract was weighed (100-500 mg). Then they were dissolved in 10 ml of distilled water in volumetric flask to prepare the solution concentrations of 10, 20, 30, 40 and 50 mg/ml respectively. 100 mg of standard reference Albendazole was dissolved in 10 ml of distilled water. Now the concentration is 10 mg/ml. Earthworms were divided into four groups, each containing three worms in Petri dish. Then roots extract was applied to the Petri dish. And one is for reference and one is for negative control.

3. Results and Discussion

3.1. Results

In our present study the cytotoxic and anthelmintic tests were conducted using *in-vitro* procedures. The *in-vitro* methods were chosen for analyzing the biological properties of the plant extracts considering their beneficial sides, like cost effectiveness and rapid

turnover, which allows the screening of the plants at large scale. Again it is known to us that different plant extracts contain a wide spectrum of bioactive compounds and these compounds are

responsible for different biological activities^[17]. In the present study, the lethality of the methanolic crude extract of root to brine shrimp was determined and the summary is expressed in Table 1.

Table 1: Cytotoxic activity of root extract of *Clerodendrum viscosum*

Sample	LC ₅₀ (µg/ml)	95% CI	Regression equation	R ²
Methanolic extract	10.235±1.061*	7.60-12.87	y = 39.25x + 10.02	0.937
Vincristine sulphate (positive control)	0.841±0.025	0.78-0.90	y = 34.02x + 52.58	0.952

*P<0.05, Statistically significant difference as compared to the standard

The lethal concentration LC₅₀ value of the test samples after 24 hour was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis (Table 1). The LC₅₀ values of the methanolic crude extract of root was found 10.235±1.061 µg/ml. However, varying degree of lethality to *C. viscosum* extract was observed with exposure to different dose levels of the test samples. The degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration (0.781 µg/ml) to highly significant with the highest concentration (400 µg/ml). Maximum mortalities took place at concentration 400 µg/ml, whereas least mortalities were at 0.781 µg/ml concentration. From the results of the brine shrimp lethality bioassay, it can be well predicted that the methanolic crude extracts possess cytotoxic principles and have moderate cytotoxic potency. It was reported by several studies that our investigational plant *C. viscosum* possesses different types of phytoconstituents like carbohydrates, glycosides, sterols and triterpenoids, tannins, saponins, alkaloids, flavonoids etc.^[18, 19]. So it may be assumed that the cytotoxic activity of the methanolic extract of roots of *C. viscosum* may be due to the presence of these phytochemicals, although the exact compound responsible for the cytotoxic activity is yet to be discovered.

Scientists have reported that several bioactive compounds such as alkaloids, tannins, terpenoids etc. are responsible for the anthelmintic activity of a plant extract^[20]. One study reported that, tannins may interfere with energy generation of worms by uncoupling oxidative phosphorylation or they binds to the free protein of the gastrointestinal tract of the worms and lead to death^[21]. In another study, alkaloids were shown to cause paralysis of the worms by acting on its central nervous system^[22]. On the other hand, the prime effect of the standard drug albendazole is to cause a flaccid paralysis of the worm which results in expulsion of the worm by peristalsis. Albendazole increases the chloride ion conductance of worm muscle membrane which produces hyperpolarization and excitability reduction that ultimately leads to muscle relaxation and flaccid paralysis of worms^[23]. It is expected that the phytochemicals present in the methanolic extract of *Clerodendrum viscosum* roots may have produced similar effects, causing death of the worms. In our study, the methanolic crude extracts and water extracts showed significant anthelmintic activity on selected worms. Methanol extracts found to be highly active. The methanol extracts demonstrated paralysis as well as death of worms in a less time as compared to water extract in case of *P. posthuma*. But water extract showed less activity on worms than Albendazole (Table 2).

Table 2: Anthelmintic activity of roots extract of *Clerodendrum viscosum*

Extract	Concentration (mg/ml)	<i>Pheretima posthuma</i>	
		P	D
ME	10	76.44±0.77*	205.31±1.3*
	20	49.28±0.60*	134.20±0.56*
	30	37.09±0.23*	105.44±0.31*
	40	21.50±0.10*	69.33±0.42*
	50	13.23±0.49*	45.10±1.79*
WE	10	181.01±0.66*	377.11±0.3*
	20	147.30±1.89*	311.55±0.97*
	30	97.06±0.90*	289.15±0.56*
	40	103.50±1.12*	232.10±1.54*
	50	78.24±0.17*	177.37±0.62*
Albendazole	20	17.67±0.54	48.00±0.47

Each value is represented as mean ± standard deviation (n = 3); *P<0.05, Statistically significant difference as compared to the standard; ME=Methanolic extract, WE=Water extract, P=Time taken for paralysis (min), D=Time taken for death of worms (min).

4. Conclusion

The pharmacological investigation for cytotoxic properties of crude extracts (methanolic extract) of *Clerodendrum viscosum* roots by using brine shrimp lethality bioassay showed a moderate cytotoxicity in comparison with positive control Vincristine sulphate. Anthelmintic properties of *C. viscosum* roots show that methanolic crude extract of roots highly consist anthelmintic properties more than water extraction. In this study, the use of roots of *C. viscosum* as an anthelmintic have been confirmed as the roots extracts displayed activity against the worms used in the

study. In conclusion, it can be suggested that the crude extracts of *C. viscosum* roots possess some action of cytotoxic and anthelmintic activities. However, further researches are necessary to find out the active principles responsible for these activities.

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6. References

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