

INVESTIGATION OF *IN-VITRO* ANTHELMINTIC AND CYTOTOXIC ACTIVITIES OF *ARTABOTRYS HEXAPETALUS* (FAMILY: ANNONACEAE) BARK GROWING IN BANGLADESH**Niaz Morshed, Md. Mizanur Rahman Moghal^{*}, Mohammad Nurul Amin, Md. Golam Kibria and Syed Masudur Rahman Dewan**Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali- 3814, Bangladesh
(*E-mail: pharmamizan@gmail.com)**ABSTRACT**

The methanolic extract of bark of *Artabotrys hexapetalus* were investigated for *in-vitro* anthelmintic and cytotoxic activities. Evaluation of cytotoxic activity was done using the brine shrimp lethality bio-assay. The crude methanolic extract showed significant cytotoxic potential (LC₅₀ value of 7.688 µg/ml) comparing with that of standard vincristine (0.839 µg/ml). The other study was undertaken to evaluate anthelmintic activity where albendazole was used as reference standard. Methanolic extract of barks (50 mg/ml) caused paralysis of the worms at 68.33 minutes and death at 84.0 minutes while albendazole (positive control) paralyzed and killed the worms at 17 minutes and 48 minutes respectively at the concentration of 10 mg/ml. The study confirms the significant anthelmintic activities of bark extract of *Artabotrys hexapetalus* and therefore demands the isolation of active principles through bioassay.

KEY WORDS: Annonaceae, anthelmintic activity, *Artabotrys hexapetalus*, cytotoxic activity**INTRODUCTION**

Among the natural sources, medicinal plants play an important role to most of the medicinal preparations as raw plant materials, refined crude extracts and mixtures etc. Even in recent times, majority of the people are still depending on the traditional medicine for their primary health care. According to the World Health Organization, almost 80% of the world's population is still relying on traditional plant-based medicines (WHO, 1993). It has been studied that fruits and herbs containing phytochemicals and non-nutritive may protect human from a host of diseases for their biological activities (Argal and Pathak, 2006). *Artabotrys hexapetalus* belongs to the family Annonaceae are a large family that contains approximately 128 genera with over 2000 species (Cronquist, 1981; Kessler, 1993). Annonaceae are morphologically highly diverse family which represent large part of plant diversity. The family generally considered to be a natural family and includes trees, shrubs and lianas, found in almost all vegetation types (Angiosperm Phylogeny Group, 2009). The flowers of *A. hexapetalus* are used in Indian subcontinent to make a stimulant tea (Ekundayo, 1989). Our main goal was to evaluate the possible cytotoxic and anthelmintic activities of the bark of *A. hexapetalus* to validate its traditional use.

MATERIALS AND METHODS**Plant materials collection and processing**

Plant samples of *A. hexapetalus* were collected from Chittagong Metropolitan Police line, Dampara, Chittagong area. The plant was identified and authenticated by an expert botanist of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession No.: 35677).

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.

Extraction of plant materials

About 370 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 2000 ml of methanol (Merck, Germany). The container with its contents was sealed and kept for a period of 20 days accompanying regular shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white filter cloth. The filtrate (methanol extract) obtained was evaporated under normal environmental condition. It rendered a gummy concentrate of reddish black color, designated as crude extract of methanol. The dry crude extracts were weighed and stored in air-tight container with necessary markings for identification and kept in refrigerator at 4°C for future investigation.

***In-vitro* cytotoxic activity**

The cytotoxic activity of the extract was evaluated using brine shrimp lethality bio-assay test (Meyer *et al.*, 1982; Mclaughlin, 1982).

Preparation of the positive control group

Positive control in a cytotoxicity study is a widely accepted cytotoxic agent and the result of the test agent is compared with the result obtained for the positive control. In the present study vincristine sulphate is used as the positive control. Measured amount of the vincristine sulphate is dissolved in DMSO to get an initial concentration of 40µg/ml from

which serial dilutions are made using DMSO to get 20 μ g/ml, 10 μ g/ml, 5 μ g/ml, 2.5 μ g/ml, 1.25 μ g/ml, 0.625 μ g/ml, 0.3125 μ g/ml, 0.15625 μ g/ml and 0.078125 μ g/ml. Then the positive control solutions are added to the pre-marked vials containing 10 living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

Preparation of negative control group

100 μ l of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as control groups.

Counting of nauplii

After 24 hours, by using a magnifying glass, the vials were inspected 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.

In-vitro anthelmintic activity

The anthelmintic activity was carried out by the method of Ajaiyeoba *et al* (2001) with minor modifications. Adult earthworms were selected for the study of anthelmintic activity because of their anatomical and physiological resemblance with the intestinal roundworm parasites of human being (Vidyarthi, 1967; Thorn *et al*, 1977; Chatterjee, 1967, Kumar BSA *et al*, 2010; Kumar A *et al*, 2010). Earthworms are widely used as effective tools for anthelmintic study due to their availability (Sollmann, 1918; Jain *et al*, 1972; Szewezuk *et al*, 2003; Dash *et al*, 2002). Adult earthworm (*Pheretima posthuma*) were collected (3-5 cm in length and 0.1- 0.2 cm in width weighing about 0.8-3.04 g) from moist soil of a road side field of Noakhali Science and Technology University, Sonapur, Noakhali. All the worms were properly washed with normal saline in order to remove all fecal materials.

Extracts were weighed and dissolved in 10 ml of distilled water to obtain the of 10, 20, 30, 40 and 50 mg/ml. Albendazole was used as reference standard (10 mg/ml). Earthworms were divided into seven groups (each containing three worms) in petridish. In five groups extract solution was applied, one is for reference and one is for negative control. Observations were made for the determination of paralysis time and death time of the worm. Paralysis was designated as the occurrence where the worms do not move even in normal saline and death was confirmed when the worms lose their motility followed with fading away of their body color.

RESULTS AND DISCUSSION

Brine shrimp lethality bioassay

LC₅₀ data of vincristine sulphate and methanolic extract has been given in table 1, figure 1 and 2. From the data, we see that the LC₅₀ value of the methanolic extract is 7.688 μ g/ml. and the vincristine sulphate showed LC₅₀ at concentration of 0.839 μ g/ml. From the results of the brine shrimp lethality bioassay it can be well predicted that the methanolic extract possess cytotoxic principles.

Table 1: Cytotoxic effect of the test sample of *A. hexapetalus*

Sample	LC ₅₀ (μ g/ml)	Regression equation	R ²
Vincristine sulfate (positive control)	0.839	y = 34.02x + 52.58	0.952
Methanolic extract	7.688	y = 30.40x + 23.07	0.947

Table 2: Anthelmintic activity of crude methanolic extract of bark of *Artabotrys hexapetalus* against *Pheretima posthuma*.

Group	Concentration (mg/ml)	Paralysis time (min.)		Death time (min.)	
		Mean \pm S.E.M.		Mean \pm S.E.M.	
Sample I	10	94.67 \pm 0.98		125 \pm 1.65	
Sample II	20	88.33 \pm 1.186		104.67 \pm 1.18	
Sample III	30	83 \pm 0.98		102.67 \pm 0.98	
Sample IV	40	76.33 \pm 1.186		94.67 \pm 1.65	
Sample V	50	68.33 \pm 0.98		84 \pm 1.24	
Standard	10	17.67 \pm 0.54		48 \pm 0.47	

In-vitro anthelmintic activity

From the data (Table 2), we see that, the methanolic extract of *Artabotrys hexapetalus* demonstrated paralysis as well as death of worms in a much more time even in higher concentration of 50 mg/ml (paralysis and death time was 68.33 ± 0.98 minutes and 84 ± 1.24 minutes) as compared to albendazole especially at lower concentration of 10 mg/ml (paralysis and death time was 17.67 ± 0.54 minutes and 48 ± 0.47 minutes). And at concentration 10mg/ml the sample have average paralysis and death time of 94.67 ± 0.98 minute and 125 ± 1.65 minute.

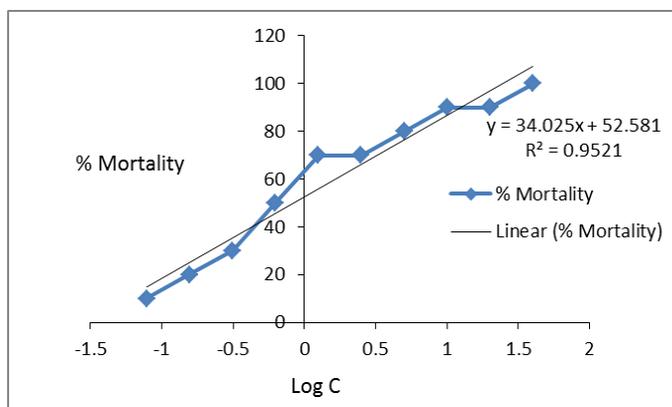


Figure 1: Effect of vincristine sulfate on shrimp nauplii.

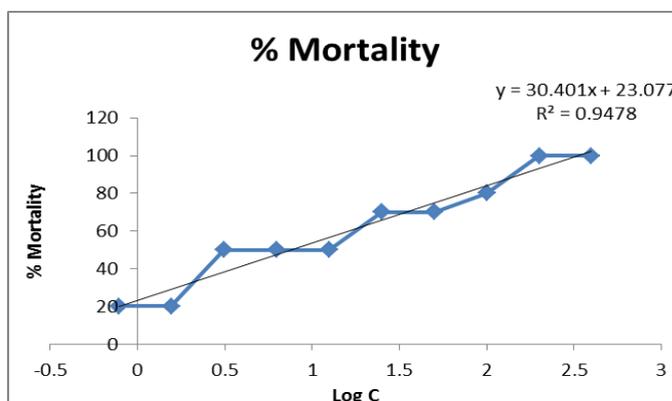


Figure 2: Effect of methanolic extract on shrimp nauplii

CONCLUSION

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticated research is necessary to reach a concrete conclusion about the findings of the present study.

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