

## Anthelmintic and Cytotoxic Activities of Two Medicinal Plants: *Polygonum viscosum* and *Aphanamixis polystachya* Growing in Bangladesh

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### Abstract

The present study was designed to investigate *in vitro* anthelmintic and cytotoxic activities of crude methanolic extract of two plants (*Polygonum viscosum* and *Aphanamixis polystachya*) grown in Bangladesh. Evaluation of cytotoxic activity was done using the brine shrimp lethality bioassay. The crude methanolic extract of *Polygonum viscosum* showed significant cytotoxic potential (LC<sub>50</sub> value of 6.34 µg/ml) among all the fractions comparing with that of standard vincristine sulphate (0.825 µg/ml). Besides, the LC<sub>50</sub> values of crude methanolic extract, pet ether and chloroform extracts of *Aphanamixis polystachya* showed good cytotoxic activities 11, 10.36, and 16.45 µg/ml, respectively. The other study was undertaken to evaluate anthelmintic activity (using *Pheretima posthuma* model) where piperazine was used as reference standard. The crude methanolic extract of *Polygonum viscosum* leaves produced a significant anthelmintic activity in dose dependent manner and the activity of crude extract was comparable with that of standard drugs. Besides, the *Aphanamixis polystachya* extract revealed moderate anthelmintic activity. Here, the anova testing was done with the  $P < 0.05$ . Further studies are suggested to determine the active compounds responsible for the anthelmintic and cytotoxic activities of these two plant extracts.

**Keywords:** Anthelmintic; Cytotoxic; Medicinal plant; *Aphanamixis polystachya*; *Polygonum viscosu*.

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### 1. Introduction

Nature is a nice source of salvation for human being by providing different remedies from its plants, animals and other sources to treat all ailments of mankind. Among all of the natural sources, medicinal plants are important contributors to the medicinal preparations. Several thousands of plants containing medicinal values have been identified by expert scientists for treating different ailments [1]. So, medicinal plants always play an important and crucial role for the development of health in mankind. According to WHO (World

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Health Organization), 80% of world's population is reliant on medicinal plants to maintain their health and to cure their ailments [2].

*Polygonum viscosum* (belonging to the family polygonaceae) is an annual herb native to Nepal and also widely distributed in Bangladesh, northeast India, Japan and China [3]. The genus *Polygonum* is distributed throughout the world, mostly in north temperate regions [4]. This genus is well known for its application in the oriental traditional medicine systems for the treatment of various disorders such as fever, pain, infections, inflammation, cancer and tumour [5]. *Aphanamixis polystachya* belonging to the family Meliaceae is another traditional plant containing potential medicinal values [6]. This medicinal plant is distributed in Bangladesh [7]. *Aphanamixis polystachya* (*A. polystachya*) stem barks are used traditionally for treating tumours, cancer, spleen diseases, rheumatism at Bengal, southeast and north region. The fruits of this plant are applied as anthelmintic and laxative. It is said also to be useful against ulcers and rheumatism [8].

According to our knowledge, there are very few studies are presently available that documents the biological activities of *polygonum viscosum* (*P. viscosum*) and *aphanamixis polystachya* (*A. polystachya*). So, in this study, our main goal was to evaluate the biological activities like anthelmintic and cytotoxic activities of two medicinal plants to validate its use in folkloric treatments.

## **2. Materials and Method**

### ***Plant materials***

or this present investigation, the *P. viscosum* barks and *A. polystachya* leaves were collected from Comilla (Bangladesh) in December 2011. The plants were identified and authenticated by BNH (Bangladesh National Herbarium), Mirpur, Dhaka (DACB Accession No. 38305 and 38304, respectively).

### ***Chemicals and reference drug***

All the chemicals and reagents for these experiments were purchased from Sigma Chemical Co. Ltd., (St. Louis, MO, USA) and E. Merck (Germany).

### ***Preparation of plant extracts***

For methanolic extraction of a plant, almost 450 grams of air dried, powdered sample were mixed in 2500 ml of about 80% methanol (Merck KGaA, Darmstadt, Germany) in a clean and sterilized glass container. It was done individually for both plants in order to obtain methanolic extract of both plants separately. The glass containers with its contents were sealed and kept for maceration for 15 days accompanying occasional shaking and stirring. At the end of 15th day, the whole mixture of each plant was filtered carefully using filter cloth and Whatman® filter paper (Sargent-Welch, USA). The resultant filtrates were then allowed to evaporate in water bath maintained about 40°C to dryness and thus a semisolid mass of the extract was obtained individually for each plant. This gummy semisolid mass was designated as crude extract of methanol.

### ***Solvent-solvent partitioning***

Solvent-solvent partitioning was done using the protocol designed by Kupchan [9], which is slightly modified. The crude extract (5 gm) of each plant was dissolved in 10% aqueous methanol properly. Then, it was extracted with petroleum ether, then with carbon tetrachloride and finally with chloroform. Thus extract of petroleum ether, carbon tetrachloride and chloroform were obtained individually.

### ***Anthelmintic activity***

The anthelmintic study was carried out by the method of Ajaiyeoba *et al.* [10] with minor modifications. Adult earthworms were used to study the anthelmintic activity because they are anatomically and physiologically resemble with the intestinal roundworm parasites of human being [11]. They are widely used as effective tools for anthelmintic study because of their easy availability [11]. The earthworms belonging to species *pheritima posthuma* (annelida), about 3-5 cm in length and 0.1- 0.2 cm in width weighing about 0.8-3.04 g, were collected from the moist soil of Noakhali Science and Technology University, Sonapur, Noakhali and identified by the Department of Fisheries and Marine Science (FIMS), Noakhali Science and Technology University (Voucher No. 112/2013).

The crude methanolic extracts of bark of *aphanamixis polystachya* and leaves of *polygonum viscosum* were used as test samples. They were used to prepare different concentrations (10-80 mg/ml) separately. For the methanol extract different concentrations were prepared by weighing 100 mg, 200 mg, 400 mg, 600 mg and 800 mg extracts and dissolving them in 10 ml distilled water separately. 100 mg of piperazine citrate was measured by weighing machine and dissolved in 10 ml water to make a concentration of 10 mg/ml. A control group was established with distilled water to ensure that the test was a validate one. Earthworms were divided into twelve groups (each containing four earthworms) in petridish. Five groups were used for the five concentrations of methanolic extracts of stem bark of a *Aphanamixis polystachya* and another five groups for the methanolic extracts of leaves of *polygonum viscosum*. One group was applied to reference standard and another to control group. Finally, the time of paralysis and death was determined consciously. Time for paralysis was noted when no movement of any sort could be observed except when the worms were vigorously shaken. Time for death of worms was taken after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (almost 50 °C) followed with fading away of their body colors [12]

### ***Cytotoxic activity***

The cytotoxic property of the extract was determined using brine shrimp lethality test [11,13]. The investigation was done on *artemia salina* (brine shrimp). *Artemis Salina leach* (brine shrimp eggs) collected from pet shops was used as the test organism. One spoon of cyst was hatched for about 48 hrs in saline water, prepared by dissolving 20 g pure NaCl and 18 g normal edible NaCl into 1 L water. The hatched cyst in turn became living nauplii. Different concentrations of the extract were prepared using dimethyl sulfoxide (DMSO) as solvent. For the test, different concentrations of plant extract prepared were added to test tubes, each containing 10 shrimps in saline water. Here,

vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an primary concentration of 40µg/ml from which serial dilutions were made using DMSO to get 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.3125 µg/ml, 0.15625µg/ml and 0.78125µg/ml solution from the extract. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

### **Counting of Nauplii**

After 24 hours, the vials were inspected by using a magnifying glass and the number of survived nauplii in each vial was counted consciously. From this result, the percent (%) of lethality of the brine shrimp nauplii was calculated nicely for each concentration.

## **3. Results**

### **Anthelmintic activity**

The crude methanolic extracts of *polygonum viscosum* leaves produced a significant anthelmintic activity in dose dependent manner and the activity of crude extract was comparable with that of standard drugs (Table 1 and Fig. 1). Besides, the *Aphanamixis polystachya* barks revealed moderate anthelmintic activity. Here, the anova testing was done with the *P* value < 0.05.

Table 1. Evaluation of anthelmintic Activity of mmethanolic extracts of *polygonum viscosum* and *aphanamixis polystachya*.

Test substance	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (distilled water)	10	-	-
Standard (piperazine)	10	56.20±0.200	71±0.244
Methanolic extract of <i>Polygonum viscosum</i>	10	6.09±1.652	9.98±0.645
	20	5.14±0.912	8.35±0.851
	40	2.84±0.629	3.97±1.291
	60	0.89±2.101	1.40±1.452
	80	0.70±0.200	1.23±0.250
Methanolic extract of <i>Aphanamixis polystachya</i>	10	92.25±1.650	146.25±0.646
	20	84.25±0.913	104.5±0.854
	40	81.75±0.629	93.76±1.25
	60	74±2.102	89.5±1.490
	80	69±0.200	81±0.244

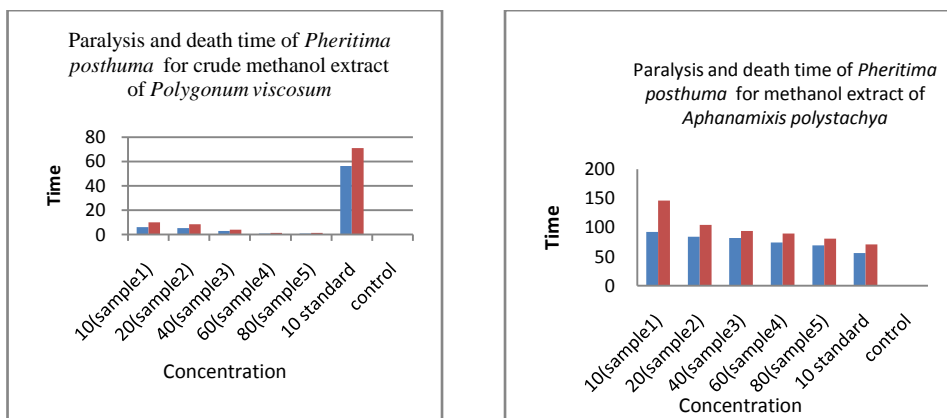


Fig. 1. Paralysis and death time bar diagram of *pheritima posthuma* for crude methanolic extract of *polygonum viscosum* and *aphanamixis polystachya*.

### Cytotoxic activity

The  $LC_{50}$  values of crude methanol extract, pet ether, chloroform and Carbon tetra chloride soluble fraction of *Polygonum viscosum* found to be 6.34, 14.12, 36.12 and 22.66  $\mu\text{g/ml}$ , respectively (Table 2). Besides, the  $LC_{50}$  values of crude methanol extract, pet ether, chloroform and carbon tetra chloride soluble fraction of *Aphanamixis polystachya* found to be 11, 10.36, 16.45 and 104.8  $\mu\text{g/ml}$ , respectively (Table 3). The positive control vincristine sulphate showed  $LC_{50}$  at a concentration of 0.825  $\mu\text{g/ml}$

From the results of the brine shrimp lethality bioassay of *Polygonum viscosum* leaves extracts it can be well predicted that the crude methanolic extract possess cytotoxic principles and the three other fractions have less cytotoxic potency. Crude methanolic extract showed the highest potency ( $LC_{50}$  6.34  $\mu\text{g/ml}$ ) among all the fractions. For *Aphanamixis polystachya* barks extracts it can be well predicted that the carbon tetra chloride extract soluble fractions have less cytotoxic potency and three others possess good cytotoxic principles.

Table 2. Results of different samples of *polygonum viscosum* leaves.

Sample	$LC_{50}$ ( $\mu\text{g/ml}$ )	Regression equation	$R^2$
Vincristine sulphate	0.825	$y=34.02x+52.58$	0.952
Methanolic crude extract	6.34	$y=31.40x+24.82$	0.917
Chloroform extract	36.12	$y=32.21x-0.178$	0.969
Carbon tetra chloride extract	22.66	$y=37.04x-0.204$	0.982
Pet. ether extract	14.12	$y=30.8x+14.58$	0.957

Table 3. Results of the samples of *Aphanamixis polystachya*.

Sample	LC50 ( $\mu\text{g/ml}$ )	Regression equation	$R^2$
Vincristine sulphate	0.825	$y=34.02x+52.58$	0.952
Methanolic crude extract	11	$y= 34.02x + 14.56$	0.982
Chloroform extract	16.45	$y=32.01x+11.07$	0.971
Carbon tetra chloride extract	104.8	$y=27.17x -4.899$	0.970
Pet. ether extract	10.36	$y=25.76x +23.85$	0.985

#### 4. Discussion and Conclusion

Medicinal plants have a long history of serving people in many regions of the world. Report shows that almost 80% of the world population still uses medicinal plants for various medical purposes [14]. It is well known that pharmaceutical companies throughout the world are trying to develop more effective drugs to treat pain, fever and so on. [1]. In the current study, two *in vitro* tests of plant were performed. One of the major advantages of evaluating the biological activities of plant extracts *in vitro* is that, the process is cost effective and includes rapid turnover allowing the screening of any plant at large scale [15]. Here, the extracts of *Polygonum viscosum* leaves showed a significant anthelmintic activity in dose dependent manner and the extracts of *Aphanamixis polystachya* barks revealed moderate anthelmintic activity. Actually, the phyto-constituents like alkaloids, tannins, phenols etc. may be responsible for anthelmintic activity [15]. It was reported that, tannins may interfere with energy generation of worms by uncoupling oxidative phosphorylation and lead to death. Besides, alkaloids were also reported to cause paralysis of the worms by acting on its central nervous system. On the other hand, The major effect of anthelmintic drug is to cause a flaccid paralysis of the worm [15]. Therefore, in our study, it is clear that these two plants showed similar effects like anthelmintic drugs against *Pheritima postuma*.

These plants also revealed good cytotoxic potency. It is said that plant extracts containing a higher concentration of bioactive compounds show cytotoxic activity. Flavonoids, which can be referred to as nature's biological response modifiers shows anti-allergic, anti-inflammatory, antimicrobial, and anticancer activities. Some studies tend to suggest that tannins may contain significant cytotoxic and antitumor potency [2].

In the light of the results of the present study, it can be summarized that the plant extracts of *Polygonum viscosum* and *Aphanamixis polystachya* possess several phytochemicals like alkaloids, tannins, phenols, flavonoids and saponins etc. which may be accountable for the possible anthelmintic and cytotoxicity activity.

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