

***In vitro* Anthelmintic and Cytotoxic Activities of
Methanolic Bark Extract of *Mimusops elengi* Linn.**

Mahmuda Nasrin, Pritesh Ranjan Dash and *Moni Rani Saha

Department of Pharmacy, Stamford University Bangladesh
51, Siddeswari Road, Dhaka-1217, Bangladesh.***Corresponding Author**Mrs. Moni Rani Saha
Assistant Professor,
Department of Pharmacy
Stamford University Bangladesh
51, Siddeswari Road
Dhaka-1217, Bangladesh.
Contact no.: +880 1711 013 960
E-mail: saha_moni1978@yahoo.com

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ABSTRACT

The methanolic bark extract of *Mimusops elengi* Linn. (Family, Sapotaceae) was screened for *in vitro* anthelmintic activity against earthworms (*Pheretima posthuma*) and cytotoxic activity by brine shrimp lethality bioassay. The extract showed anthelmintic activity at all the concentrations of 25mg/ml, 50mg/ml and 100mg/ml. But the potent anthelmintic activity was observed at the concentration of 100 mg/ml. At this concentration, the time required for paralysis and death of earthworms was about 22 and 34 minutes respectively whereas time taken for paralysis and death by the standard drug Pyrantel pamoate at 10mg/ml was about 26 and 38 minutes respectively. The extract also exhibited good cytotoxic activity with LC₅₀ value of 40µg/ml whereas LC₅₀ of vincristine sulphate was 0.078µg/ml. The experimental results suggest that *Mimusops elengi* Linn has both *in vitro* anthelmintic and cytotoxic activities.

Key word: *Mimusops elengi*, anthelmintic, cytotoxic, earthworm.**INTRODUCTION**

Medicinal plants as a whole or their parts are being used as antibacterial and anthelmintic, anti-inflammatory etc. since the time immemorial. Now-a-days, the medicines available in the market from which most of them either not effective up to the mark or resistance has been developed which results in reoccurrence again. Plants can be a better alternative as they have nutritive value as well as possess number of pharmacological activities with fewer side effects (Greathead, 2003). Plant derived drugs serve as a prototype to develop more effective and less toxic medicines (Rastogi et al., 2009).

Mimusops elengi Linn. commonly known as Bakul (Bengali) belongs to the family Sapotaceae and is a small to large evergreen tree found all over the different parts of Bangladesh, Pakistan and India (Ghani 2003). It has been used in the indigenous system of medicine for the treatment of various ailments. Several therapeutic uses such as cardiogenic, alexipharmic, stomachic, anthelmintic and astringent have been ascribed to the bark of *Mimusops elengi* Linn. (Ghani, 2003; Kirtikar and Basu, 1935) The bark and fruit of this plant are used in the treatment of diarrhea and dysentery, and a decoction of the bark is used as a gargle (Jahan et al., 1995). Phytochemical review shows that the bark of this plant contains taraxerol, taraxerone, ursolic acid, betulinic acid, V-spinosterol, W-sitosterol, lupeol (Misra and Mitra, 1967; Misra and Mitra, 1968), alkaloid isoretronecyl tiglolate (Hart et al., 1968) and mixture of triterpenoid saponins. In this study, the anthelmintic and cytotoxic activities of the crude bark extract have been reported.

MATERIALS AND METHODS**Drugs and Chemicals**

Pyrantel pamoate and Normal saline water were used during the experiment. Methanolic extract of barks of *Mimusops elengi* Linn. was tested in various doses in each group. Normal saline water was used as control. Pyrantel pamoate collected from Beximco Pharmaceuticals Ltd. was used as the standard drug for comparative study in the *in vitro* anthelmintic activity.

Earthworms

Adult earthworms (*Pheretima posthuma*) were used to evaluate anthelmintic activity by *in vitro* method. Earthworms were collected in IUT in Gazipur district and identified in the Pharmacology Laboratory of the Department of Pharmacy, Stamford University Bangladesh. The earthworms were 3-5 cm in length and 0.1-0.2 cm in width.

Collection and Identification of plants

Mimusops elengi Linn. was collected from the district of Gazipur. The plant was collected and identified in Bangladesh National Herbarium Mirpur, Dhaka and accession number for this plant was 34,486. The plant part was thoroughly washed with water, cut into small pieces and dried in the sun.

Extraction

After drying the bark was reduced to coarsely powder using a grinding mill. 93 gm powder was extracted with a mixture of methanol: water (8:2, v/v) by a Soxhlet apparatus at 60°C. The solvent was completely evaporated and obtained 29gm (yield = 31.23%) dried crude extract which was used for *in vitro* anthelmintic and cytotoxic investigations.

Phytochemical Screening

The freshly prepared methanolic extracts of the plant were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: alkaloids with Dragendorff's, Hager's and Mayer's reagent, glycosides with NaOH, glucosides with Fehling's reagent and HCl, flavonoids with the use of Zinc ribbon and HCl; tannins with ferric chloride and potassium dichromate solutions, steroids with sulfuric acid and saponins with ability to produce suds. Carbohydrate was tested using Molish reagents and Fehling's reagent. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

Anthelmintic Activity

The Anthelmintic assay was carried out as per the method described by Ajaiyeoba et al., 2001. The assay was performed *in vitro* using adult Bangladeshi earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation for its anthelmintic activity. Test samples of the extract were prepared at the concentrations of 25, 50 and 100mg/ml in normal saline water and six earthworms (*Pheretima posthuma*) approximately equal size (same type) were placed in each beaker containing 50ml of above test solutions of extract. Pyrantel pamoate (10mg/ml) was used as reference standard and normal saline water as control. All the test solutions and standard drug solution were prepared freshly before starting the experiments. The time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously (Kumanan et al., 2010). Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C).

Cytotoxicity study

Brine shrimp lethality bioassay (Meyer et al., 1982) technique was applied for the determination of cytotoxic property of methanolic bark extract of *Mimusops elengi* Linn. For Brine Shrimp lethality bioassay the eggs of Brine Shrimp (*Artemia salina*, Red Top®, Ocean Star International, USA) were hatched in a tank at a temperature around 37°C equipped with constant oxygen supply for 24 hours. Stock solutions of the samples were prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). Fifty four clean vials were taken; eighteen of these were for the extract in nine concentrations (two vials for each concentration) and the rest thirty six vials for negative and positive control test.

Preparation of test samples

4ml of seawater was given to each of the vials. Then with the help of micropipette specific volumes of samples were transferred from the stock solutions to the vials to get final sample concentrations of 1.25, 2.5, 5, 10, 20, 40, 80, 160 and 320µg/ml. The concentration of DMSO in these vials should not exceed 40µl per 4ml of Brine Shrimp nauplii because above this concentration DMSO may become toxic to the nauplii.

Preparation of positive control group

Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 20µg/ml from which serial dilutions were made using DMSO to get 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.3125µg/ml, 0.15625µg/ml, 0.078125µg/ml and 0.0390µg/ml. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 4ml simulated sea water to get the positive control groups.

Preparation of negative control group

30µl of DMSO was added to each of three premarked glass vials containing 4ml of simulated seawater and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test was considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compounds.

Counting of nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted.

RESULTS

Phytochemical screening of the methanolic bark extract of *Mimusops elengi* Linn. revealed the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, glycosides, glucosides and steroids.

Anthelmintic activity

The methanolic bark extract of *Mimusops elengi* Linn. showed potent anthelmintic activity which was comparable to the standard drug Pyrantel pamoate (10 mg/ml). The results are shown in Table 1. The extract showed the most potent anthelmintic activity at the dose of 100 mg/ml at which paralysis and death of earthworms occurred within about 22 and 34 minutes respectively whereas time required for paralysis and death of earthworms by pyrantel pamoate were about 26 and 38 minutes respectively.

Table 1: Anthelmintic activity of *Mimusops elengi* Linn.

Animal Group	No. of worms	Concentration	Time taken for paralysis (min)	Time taken for death (min)
Control	6	50 ml	-	-
Standard	6	10 mg/ml	26±4	38±2
Group-I	6	25 mg/ml	30±2	49±5
Group-II	6	50 mg/ml	29±5	39±4
Group-III	6	100 mg/ml	22±2	34±5

All values represent Mean ± SD; n=6 in each group. Standard: Pyrantel pamoate

Cytotoxicity study

Table 2 shows the results of brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate. The LC₅₀ obtained from the best-fit line slope was found to be 40µg/ml for methanolic crude extract of the bark of the plant and 0.078 for vincristine sulphate.

Table 2: LC₅₀ data of the test samples of *M. elengi* Linn. in brine shrimp lethality bioassay

Plant parts	Crude extracts	LC ₅₀ (µg/ml)
Barks	Methanol	40
Standard	Vincristine sulphate	0.078

DISCUSSION

Parasitic worm infections of the gastrointestinal tract of human beings and animals have been renowned to have adverse effects on health standards with a consequential lowering of resistance to other diseases. To evaluate compounds with anthelmintic activity, a number of substances were analyzed using different species of worms, for example, earthworms, ascaris, nipostromylus and heterakis. From all these species, earthworms have been used extensively for the preliminary evaluation of anthelmintic compounds *in vitro* because they are similar to intestinal "worms" in their reaction to anthelmintics and are easily accessible. It has been verified that all anthelmintics which are toxic to earthworms are creditable to study as an anthelmintic (Sollmann, 1918). Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. From literature review and phytochemical analysis it was found that the plant, *Mimusops elengi* Linn. contains phenolic compounds. Tannins which are polyphenolic compounds were known to have anthelmintic activities (Bate-Smith 1962; Niezen et al., 1995) and it is due to binding of tannins to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may be responsible for death (Thompson et al., 1995; Athnasiadou et al., 2001). Also some flavonoids and other secondary metabolites are known to have anthelmintic activities but exact mechanisms are not clearly established. (Kerboeuf et al., 2008).

In the current study brine Shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer activity of natural products. It is indicative of cytotoxicity and a wide range of pharmacological activity of the compounds (Persoone 1980; Meyer et al.1982). As compared to positive control vincristine sulphate, the bark extract exhibited considerable cytotoxic activity. As phytochemical tests showed the presence of alkaloids in methanolic extract, it might be responsible for cytotoxic effect. This finding clearly indicates the presence of bioactive principles in this crude extract which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents.

CONCLUSION

The results of the present study reveal that the bark of *Mimusops elengi* Linn. has *in vitro* anthelmintic and cytotoxic activities which can be further evaluated to establish its therapeutic value as well as its mechanism of action after compound isolation.

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