

Investigations of Anthelmintic Activity of Ethanolic Leaf Extract of *Premna esculenta*

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Abstract

The establishment of anthelmintic resistance and the high cost of conventional anthelmintic drugs led to the assessment of medicinal plants as an alternative source of anthelmintic using the *P. posthuma* mature earthworm. The timing of the earthworms paralysis and death at (25, 50, and 100 mg/ml) of three different concentrations was investigated. There was dose-dependent action in the plant extract, however, the ethanolic extract showed more than the aqueous extract. Albendazole was used as a standard reference at the same concentration as an extract, and normal saline served as the control. As compared to the conventional reference drug albendazole, compared to pure water, albendazole (10 mg/ml) is utilized as the standard reference medication. This is revealed by the results of the current study.

Keywords: *P. posthuma* • Anthelmintic • Albendazole

Introduction

Anthelminthics, also known as anthelmintics, are medicines that kill or stupefy parasitic worms (helminths) so they may be eliminated from the body [1]. According to the WHO, an astounding two billion individuals are infected with parasitic worms. Also affecting cattle and crops are parasitic worms that have an economic impact by altering food production. Despite the high frequency of parasitic illnesses, anthelmintic medication research is lacking. According to WHO, only a select few medications are used routinely utilized to treat certain parasite disorders. Traditional medicine claims that several natural herbs are effective at getting rid of worms [2].

Tribal people have historically treated a variety of inflammatory disorders with *P. esculenta* (Family: Verbenaceae). A shrub with short limbs and branching is known as *P. esculenta* Roxb. Family Verbenaceae and is found in the forest areas of Bangladesh and the Chittagong and Chittagong Hill Tracts of India [3]. The herb often referred to as "Lelompata," has long been used by tribal people in Bangladesh to cure Jock worm and appetite infection, frenzy, hepatic, yellow fever, fluor albus, tumor, swelling, serpent bite, abdomen ailments, and kidney stones. Consequently, for the treatment of bacterial and fungal infections, arthritis, and other conditions, the plant's leaves are applied directly to the area. That is afflicted in traditional medicine. Roots are frequently combined with other plants to treat gout, edema, and jaundice. The leaves are one of the ingredients of a treatment used to cure jaundice in Khagrachari, Bangladesh. For jaundice patients in the Chittagong Hill Tracts, leaves boiled with a Nappi fermented paste made from different marine fish species are a crucial part of their diet [4].

Materials and Methods

The experiment used two substances: pyrantel pamoate and ordinary saline

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water. In each group, different dosages of ethanol extract from *P. esculenta* leaves were investigated. The control was regular saline water. The reference medication for the comparison investigation in the *in vitro* anthelmintic activity was pyrantel pamoate obtained from Beximco Pharmaceuticals Ltd.

Earthworms

In vitro, testing of the anthelmintic action was done using adult earthworms (*P. posthuma*). A collection of earthworms was made at the IUT in the Gazipur area and were identified at Primeasia University in Bangladesh. The length and breadth of the earthworms were 2-4 cm and 0.2-0.3 cm, respectively.

Collection and identification of plant

The district of Gazipur is where *P. esculenta* was collected. The accession number for this plant was DACB78766, and it was collected and identified at the Bangladesh National Herbarium in Mirpur, Dhaka. The plant component was properly cleaned with distilled water, and then it was divided into little pieces and dried in the shade.

Preparation of fresh leaves extract

Weighting 75 g of the collected leaves, 150 ml of distilled water was added to make a liquid. The mixture was then centrifuged at a speed of 150 rpm. The supernatant was filtered via sterile filter paper into a conical flask since the research extract 1 ml of filtrate is expected to contain 0.5g or 500 mg/ml.

Phytochemical screening

Using established phytochemical techniques, the freshly obtained crude extract was qualitatively examined for the presence of several phytochemical elements such as alkaloids, tannins, reducing sugar, flavonoids, steroids, terpenoids, and saponins [5,6].

Anthelmintic activity

According to the procedure outlined by Ajaiyeoba EO, et al. the anthelmintic test was conducted [7]. Based on its morphological and physiological similarity to intestinal roundworm a parasite in people, the adult Bangladeshi earthworm (*P. posthuma*) was used in the experiment *in vitro* to test for anthelmintic activity. Six earthworms (*P. posthuma*) of roughly comparable size were introduced in every beaker with 50ml of the aforementioned tests for the extract solutions. The test samples of the extract were made at concentrations of 25, 50, and 100 mg/ml in normal saline water. Pyrantel pamoate (10mg/ml) served as the control and ordinary saline water as the reference standard. Before beginning the tests, all of the test solutions and the reference medication solution were newly made. When there was no movement visible other than when the worms were violently agitated, the duration of paralysis was recorded [8]. After determining that worms

Table 1. Result of phytochemical screening of *P. esculenta*.

Extract	Alkaloids	Tannins	Reducing Sugar	Flavonoids	Steroids	Saponins	Terpenoids
FLPE	+++	+++	-	++	-	+	-

FLEPE: Fresh Leaves of *P. esculent*
Present: (+); Absent (-)

Table 2. Result of *in vitro* evaluation of the anthelmintic activity of *P. esculenta*.

Treatment	Concentration (mg/ml)	<i>P. esculenta</i> Extract	
		Paralyzing Time (min)	Death Time (min)
Distilled water	-	-	-
Albendazole	10	27±0.05	68.95±0.41
	25	55±3	292±10
	50	47±4	314±12
	100	50±1	240±15
Aqueous extract	25	50±5	70±5
	50	31±5	50±5
	100	27±5	47±5

when violently shaken or submerged in warm water, remained still, the time of worm death was recorded (50°C)

caused by tannins attaching to free proteins on the cuticle of the parasite in the gastrointestinal system of the host animal and may result in the parasite's death [10,11].

Results

P. esculenta's ethanolic leaves extract underwent phytochemical screening, which identified alkaloids, tannins, reducing sugar, flavonoids, steroids, terpenoids, and saponins.

Anthelmintic activity

The ethanolic leaf extract of *P. esculenta* showed potent anthelmintic action that was equivalent to the prescribed medication Pyrantel pamoate (10 mg/ml). Results are displayed in Table 2. According to the extract, the most potent anthelmintic activity at the dose of 100 mg/ml at which paralysis and death of earthworms occurred within about 40 and 55 minutes respectively whereas the duration before earthworms get paralyzed and die by pamoate was about 30 and 60 minutes respectively. The results of phytochemical screening and *in vitro* anthelmintic activity of *P. esculent* of the fresh leaves extract are shown in (Table 1) and (Table 2) respectively.

Discussion

It is well known that parasitic worm infections of the digestive system in both people and animals have negative health outcomes and decreased resistance to other illnesses as a result. Various chemicals were examined using worm species, including earthworms, *Ascaris*, *nippostrongylus*, and *tetrakis*, to evaluate compounds with anthelmintic action. Earthworms have been utilized often from among all of these species for the first *in vitro* testing of anthelmintic compounds because of their accessibility and similarity to intestinal "worms" in terms of how they respond to anthelmintics. It has been shown that any anthelmintics that are harmful to earthworms can be considered anthelmintics [9]. Earthworms can move through ciliary movement. A mucilaginous layer made of intricate polysaccharides makes up the earthworm's outer layer. The earthworm may travel freely since this layer is slimy. Any injury to the mucopolysaccharide membrane will expose the outer layer, which inhibits its motion and may result in paralysis. By harming the mucopolysaccharide layer, this approach may result in the death of the worm. In that one literature review phytochemical analysis, it was found that the plant, *P. esculenta* contains phenolic chemicals which are

Conclusion

P. esculenta extract shows *in vitro* anthelmintic activity, according to the current investigation results. After compound isolation, this bark's medicinal efficacy and mechanism of action may be further assessed.

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