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### *In Vitro* Anthelmintic Activity Leaves Of *Morus alba* Linn. Against *Pheretima posthuma*

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#### Abstract

The present study was undertaken to evaluate anthelmintic activity of leaves of *Morus alba* Linn (Family - Moraceae) and its alcoholic, petroleum ether and aqueous extract against *Pheretima posthuma*. Various concentrations (10-50 mg/ml) of alcoholic, petroleum ether and aqueous extracts were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate was used as standard anthelmintic drug and distilled water was used as control. The results of present study indicated that the alcoholic, petroleum ether and aqueous extract significantly exhibited paralysis ( $P < 0.01$ ) in worms in doses (10, 25 and 50 mg/ml) and also caused death of worms especially at higher concentration of 50 mg/ml, as compared to standard drug.

**Key Words:** Anthelmintic, *Morus alba*, *Pheretima posthuma*, Piperazine citrate.

#### 1. Introduction

*Morus alba* Linn (Moraceae) is also known as Tut in India. The white mulberry has a long history of medicinal use in Chinese medicine; almost all parts of the plant are used in one way or another. Recent research has shown improvements in elephantiasis when treated with leaf extract injections and in tetanus following oral doses of the sap mixed with sugar. The fruit has a tonic effect on kidney<sup>1,2</sup>. It is used in the treatment of urinary incontinence, dizziness, tinnitus, insomnia due to anemia, neurasthenia, hypertension, diabetes, premature graying of the hair and constipation in the elderly<sup>3</sup>. The leaves are showing analgesic and anti-inflammatory activity of hydroalcoholic extract of leaves<sup>4</sup>. Phytochemical review shows the presence of tannins, Vitamin A, flavonoid, thiamine, protein, carbohydrates<sup>5</sup>. It has been used in the indigenous system of medicine for cooling, acrid, purgative, diuretic, laxative, anthelmintic, brain tonic, antibacterial, hepatopathy properties. They are useful in vitiated condition of *vata* and *pitta*, burning sensation<sup>6</sup>. The present study was therefore undertaken to evaluate in vitro anthelmintic activity of crude extract of *Morus alba* (90% alcoholic extract) and its different extracts against *Pheretima posthuma*.

## 2. Materials and Methods

### 2.1 Plant Collection and Authentication

The leaves of *Morus alba* Linn. were collected from Ramling mudgad Dist.-Latur (Maharashtra) and its botanical identification was confirmed from Indian Council Of Medical Research, Belgaum (Karnataka) India.

### 2.2 Preparation of Extracts

The plant material (leaves) were dried for several days and powdered with the help of an electric grinder. It was air dried and was successively extracted with petroleum ether, alcohol (90%) and finally leaves were macerated with water. The yields obtained of the different successive extract were 5.102%, 8.231% and 11.31% respectively.

### 2.3 Worms Collection and Authentication

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil were identified at the Dept. of Zoology RLS College, Belgaum.

### 2.4 Preparation of test sample

Samples for in-vitro study were prepared by dissolving and suspending 2.5 gms of each extract (alcoholic, petroleum ether and aqueous) in 25 ml of distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 25 and 50 mg/ml.

### 2.5 Anthelmintic assay

The anthelmintic assay was carried as per the method of Ajayieoba E. O. et al with minor modifications<sup>7</sup>. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings<sup>8, 9</sup>. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug<sup>10,15</sup>. The 50 ml formulations containing four different concentrations, of each alcoholic, petroleum ether, and aqueous extract (10, 25 and 50mg/ml in distilled water) were prepared and six worms (same type) were placed in each group. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C<sup>16,17</sup>. Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control.

## 3. Results and Discussion

Preliminary phytochemical screening of leaves of *Morus alba* has shown the presence of tannins, resins, flavonoids. An aqueous and alcoholic extract of *Morus alba* leaves exhibited anthelmintic activity in dose dependant manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration (Table-1). The alcoholic extract caused paralysis at 5.05min. and time of death at 31.80 min. while petroleum ether and aqueous extracts revealed paralysis at 16.19 and 13.83 min and time of death at 50.27 and 40.91min. respectively against the earthworm *Pheretima posthuma*. The standard drug Piperazine citrate showed the same at 20.22 and 55.09 minutes, respectively.

**Table 1: Anthelmintic activity leaves extract of *Morus alba* Linn**

Group	Test sub.	Concentration mg/ml	Time taken for Paralysis (P) and for Death of <i>Pheretima posthuma</i> worms (D) in min.	
			P	D
A	Vehicles	-	-	-
B	Piperazine citrate	10	20.22± 0.27	55.09±0.49
C	Alcoholic extract	10	14.35± 0.32***	49.46± 0.35***
		25	10.36±0.34***	43.18± 0.70***
		50	5.057± 0.29***	31.80±0.78***
D	Petroleum ether extract	10	24.83± 0.41***	56.09± 0.58*
		25	19.42± 0.45*	52.88± 0.29*
		50	16.19± 0.49***	50.27± 0.54***
E	Aqueous extract	10	21.21± 0.40*	49.59± 0.30***
		25	18.54± 0.28*	45.70± 0.57***
		50	13.83± 0.36***	40.91± 0.33***

Values are expressed as MEAN±SEM

One way ANOVA followed by Dunnett's 't' test.

n=6 in each group. \*P<0.05, \*\*\*P<0.01.

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis<sup>18</sup>. The leaves extract of *Morus alba* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml, in shorter time as compared to standard drug Piperazine citrate. Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents contained within them. Tannins were shown to produce anthelmintic activities<sup>19</sup>. Chemically tannins are polyphenolic compounds<sup>20</sup>. It is possible that tannins contained in the extracts of *Morus alba* produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death<sup>21,22,23</sup>. The study has shown that, alcoholic extracts of *Morus alba* leaves have significantly determined anthelmintic activity.

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