

Investigation of Invitro Anthelmthic Activity of *Diospyros malabarica* (Kostel Bark)

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ABSTRACT

Herbal medicines are free from possible side effects and they are economical and easily available will be beneficial for the mankind over the years. Helminthic infections are the common infections affecting the large portion of the world's population and can be easily treated by using natural medicine. Pharmacological screening for Anthelmthic activity of the Indian medicinal plant, *Diospyros malabarica* (Ebenaceae) claimed to be used traditionally in the treatment of various ailments including helmenthiasis. Literature survey indicated that no published reports on the above said plant for anthelmthic activity. In view of this, the author aimed to carry out the extract of *Diospyros malabarica* using solvent ethanol and then plan to study the Invitro anthelmthic activity of the ethanolic extract. Time taken for paralysis, death of the worms was recorded. It was found to be statistically significant.

Keywords: *Diospyros malabarica*, soxhlation, Helminthic infections

INTRODUCTION

Herbal medicines are major sources of health care for the entire world over the centuries¹. WHO report 80% of the world population relies on the natural drugs.²⁻⁴ Helminthic infections are now identified as cause of acute as well as chronic illness among the human beings as well as cattle's. More than half of the world's population suffers from infection of one or the other and majority of cattle's suffers from worm infections⁵. Many reports claims the efficacy of several natural plants in eliminating worms⁶ keeping this in mind the present work was designed to formulate and evaluate the anthelmthic activity of polyherbal formulation.

MATERIALS AND METHODS

Materials

The plant *Diospyros malabarica* bark was collected from Tirupathi, Andhra Pradesh, India, identified and authenticated by Dr. Maddi Ramaiah Associate Professor & Head, department of pharmacognosy, Hindu college of pharmacy, Guntur, A.P. All other solvents and chemicals used were of analytical grade. Adult earthworm of the genus and species, *Pheretima posthuma*.

Preparation of Extract

The bark of the plant was washed and cleaned. Then the bark was shade dried at room temperature. Dried bark were powdered and packed in air tight container. The coarse material was subjected to successive soxhlet extraction by using ethanol solvent. The extract was concentrated under reduced pressure and stored in desiccators for complete removal of solvent. The percentage yield was calculated.

Anthelmthic investigation

P. posthuma, Indian adult earth worms were used to screen the anthelmthic activity. Earthworms were obtained from moist soil, and washed out in to normal saline water to remove all the fecal matter and waste surrounding their body, having 3-5cm in length, 0.1-0.2 cm in width, weighing 0.8-3.04 g are selected. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically^{7,8}. Indian adult earth worms were used for the in vitro anthelmthic bioassay^{9, 10} of plant ethanolic extract. The worms were divided into the respective groups containing six-earth worms in each group. All the prototypes were suspended in minimum quantity of 0.5% v/v CMC and the volume was adjusted to 10 ml with normal saline for making the concentration of 25, 50, 100 mg/ml. All the prototypes and the standard drug solution were freshly prepared before commencement of the screening. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows, vehicle (0.5% v/v CMC in normal saline), and Albendazole (25mg/ml) and prototypes (25, 50 and 100mg/ml) the anthelmthic activity was determined. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour. Six worms of about the same size per petridish were used. They were observed for their spontaneous motility and evoked responses (table 2, figure 1).

Statistical analysis

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Table 1: Preliminary Phytochemical analysis of ethanolic extract of *Diospyros malabarica*.

S. No.	Compound	Test	Ethanolic Extract
1.	Steroids	Salkowski test	+
		Liebermann- Burchard test	+
		Liebermann's test	-
		Shinoda test	-
2.	Flavonoids	Lead acetate test	+
		NaOH test	+
		H ₂ SO ₄ test	-
		Wagner test	-
3.	Alkaloids	Hager test	+
		Mayer test	+
		Dragendorff's test	+
		5% FeCl ₃ Solution	+
4.	Tannins	Acetic acid test	+
		Potassium dichromate test	+
		Dil. Iodine solution test	+
		Dil. Potassium permanganate	+
		Dil. Nitric acid test	+

“+” indicates presence; “-“ indicates absence

Table 2: Effect of Albendazole and ethanolic extract (mg/ml) of Indian medicinal plant (*Diospyros malabarica*) on Indian earth worm *Pheretima posthuman*.

Group	Treatment	Concentration	Time taken by earthworm for Paralysis (minutes).	Death (minutes)
I	Control	-	-	-
II	Albendazole	25mg/ml	20.83 ^{ns}	30.94 ^{ns}
III	DMEE	25mg/ml	130.80*	190.25*
		50mg/ml	90.58*	110.25*
		100mg/ml	100.26*	130.56*

Values are expressed in Mean \pm SEM, n=6, * P < 0.001, ** P < 0.01, *** P < 0.05
DMEE: *Diospyros malabarica* ethanolic extract

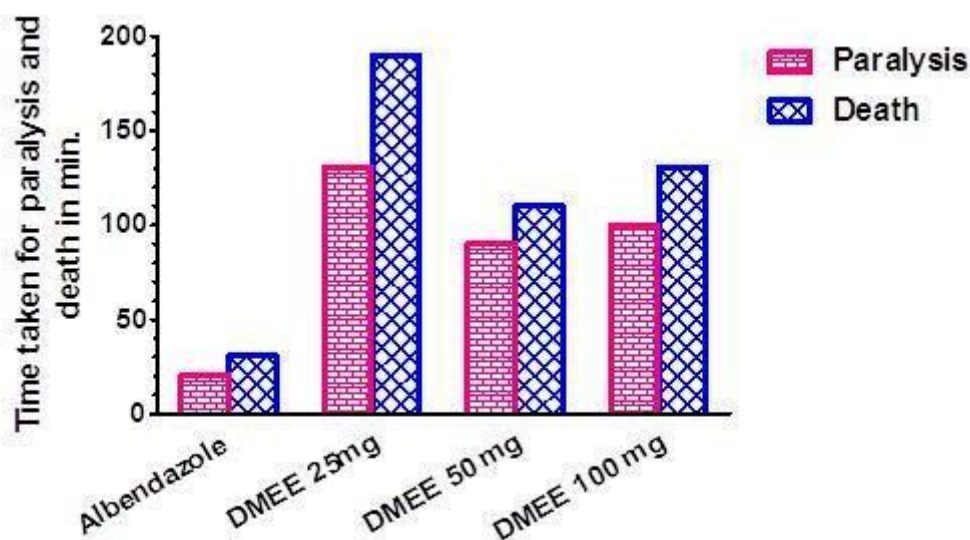


Figure 1: Effect of Albendazole and Ethanolic extract (mg/ml) of *Diospyros malabarica* on Indian earth worm *Pheretima posthuman*.

Observations were made for the time taken to paralysis and death of individual worms. Statistical evaluation of the data by Analysis of variance (ANOVA) test was performed and the results were expressed as mean \pm SEM using graph pad prism V 5.0 (n = 6). All the results were shown in Table 2 and Figure1.

RESULTS AND DISCUSSION

From the table 2 it was observed that the ethanolic extract of *Diospyros malabarica* at doses of 25 mg/ml, 50 mg/ml, 100 mg/ml concentrations paralysis and death were shown at 130.80, 90.58, 100.26 and 190.25, 110.25, 130.56

minutes post exposure respectively (table 2, figure 1). Similarly, the standard drug Albendazole also shows paralysis and death were at 20.83 and 30.94 minute post exposure respectively.

The earth worms are more sensitive to herbal ethanolic extract at 50 mg/ml concentration as compared to the reference standard Albendazole. The results were compared with the standard drug Albendazole and it was found that the herbal extract at 50 mg/ml concentration was more effective than remaining doses however it is less effective than the standard drug Albendazole (table 2, figure 1).

The effect of Albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.

The preliminary phytochemical examination of selected plant suggested that they having saponins, flavonoids, tannins, alkaloids and glycosides (Table 1). Therefore it is assuming that these phytoconstituents are responsible for anthelmintic activity. It is therefore worth study further to isolate the pure molecules responsible for anthelmintic activity.

CONCLUSION

All these scientific observations support the traditional use of *Diospyros malabarica* for treating helminthic infections.

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