

EVALUATION OF *IN VITRO* ANTHELMINTIC PROPERTIES OF *AVICENNIA MARINA* LEAVES

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## ABSTRACT

**Objectives:** The purpose of the present study was to determine the anthelmintic properties of chloroform, methanol and aqueous extracts of *Avicennia marina* leaves on Indian earthworm- *Pheretima posthuma*.

**Methods:** Three concentrations of each extract (25 mg/ml, 50 mg/ml, and 75 mg/ml) were studied in this activity to determine the time of paralysis (vermifuge) and the time of the death of the worms (vermicidal). Piperazine citrate in the same concentrations was taken a reference standard and normal water was taken as control. The time taken for paralysis and death of the worms was recorded.

**Results:** All the extracts showed dose-dependent and a significant activity was shown at higher concentration (75 mg/ml). Methanolic extract showed peak activity at a concentration of 75 mg/ml.

**Conclusion:** Based on the results it was found that the leaves of *A. marina* possess vermifuge and vermicidal activities and can be used as an effective anthelmintic.

**Keywords:** Anthelmintic, Mangroves, *Pheretima posthuma*, Vermifuge, Vermicide.

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## INTRODUCTION

Medicinal plants are known to play a key role in the management of various diseases especially in areas where affordability and availability of Western medicines are remarkably low. In fact plants have been used as therapeutic agents since ages [1]. Therefore, aiming for drugs from plants and animals is always of greater interest and continuous process for research [2]. Mangroves are special woody trees found on sheltered coast lines and river deltas, growing in brackish wetlands between land and sea where other plants cannot survive. Highly stressful habitat involving daily changes in pH of soil and water, humidity, salinity, temperature, and tidal cycles may be the possible reasons for many of these plants to synthesize enormous bioactive phytoconstituents many of which have been found to have extensive use in industry and human health care. Traditionally more than 100 numbers of mangroves and their associated plants were used in the treatment of various ailment but only a few are evaluated and documented [3]. *Avicennia marina* is one of the most common species in mangrove vegetations, commonly known as gray mangroves belongs to the family Acanthaceae (Avicenniaceae) [4]. *A. marina* has been used as traditional medicine for years with a variety of biological activities such as antioxidant, antibacterial, antifungal, antiulcer, antiplasmodial, antitumor, and anticancer properties.

Helminth infections are among the most widespread infections in humans distressing a huge population of the world [5]. In general, majority of Helminth infections are restricted to tropical regions and become a hazard to health by contributing to the prevalence of undernourishment, anemia, eosinophilia, and pneumonia [6]. Parasitic diseases cause ruthless morbidity affecting the population in endemic areas [7]. The gastrointestinal helminthes become resistant to currently available synthetic anthelmintic drugs. Hence, the need to investigate and identify natural substances with anthelmintic activity is huge [8,9]. The present study was aimed at using mangroves in the control of intestinal parasites.

## MATERIALS AND METHODS

## Collection of plant material

The plant was locally called Tella mada. The leaves of *A. marina* were collected from Coringa Wildlife sanctuary, Kakinada, Andhra Pradesh, India. The plant was botanically identified and authenticated in the Department of Botany, P.R. Government College, Kakinada.

## Preparation of extracts

Freshly collected leaf material was washed thoroughly and shade dried at room temperature, coarsely powdered and subjected to successive extraction using chloroform, methanol, and water and concentrated under vacuum to get the corresponding residues.

## Drugs and chemicals

All the chemical and reagents procured were of the Analytical grade. The drug Piperazine citrate (Glaxosmithkline) was used during the experimental protocol.

## Preliminary phytochemical screening

The preliminary phytochemical screening of the extracts revealed the presence of steroids, glycosides, flavonoids, and tannins.

## Experimental animals

Earthworms have been selected for the initial evaluation of anthelmintic activity because of their easy availability and their anatomical and physiological resemblance with the intestinal helminthes of humans [10-12].

*Pheretima posthuma* was collected from the water logged areas of soils and washed with normal saline to remove filthy matter. They were authenticated in the Department of Zoology, P.R. Government College, Kakinada.

## Ethics approval

The experiments conducted on the animals are performed in accordance with the approval of Institutional Animal Ethics Committee

Table 1: Each value represents mean±SD (n=6)

Group	Concentration (mg/ml)	Time taken for paralysis (P) in min	Time taken for death (D) in min
Control (water)	-	-	-
Chloroform leaf extract	25	42.63±0.98	107.16±1.64
	50	35.27±0.57	72.90±1.63
	75	15.45±0.46	65.90±0.64
Methanolic leaf extract	25	35.92±0.44	52.37±1.82
	50	25.31±0.65	41.54±1.05
	75	13.98±0.94	33.49±0.69
Aqueous leaf extract	25	62.15±1.06	108.83±2.49
	50	35.86±0.60	81.59±0.77
	75	24.66±0.69	74.79±0.61
Standard (Piperazine citrate)	25	30.61±0.66	53.56±0.86
	50	26.58±0.93	28.55±0.79
	75	20.53±0.75	18.52±0.97

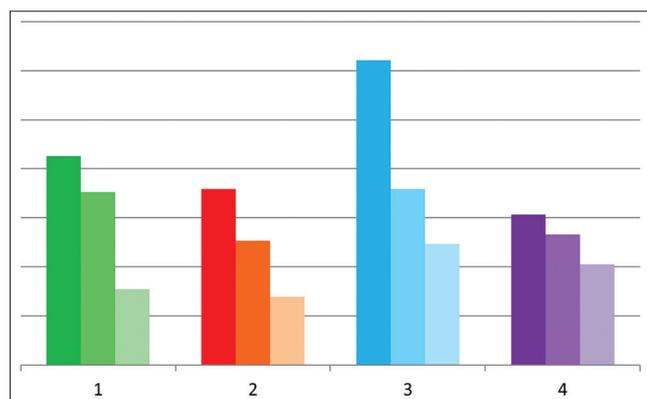


Fig. 1: Dose-dependent activity

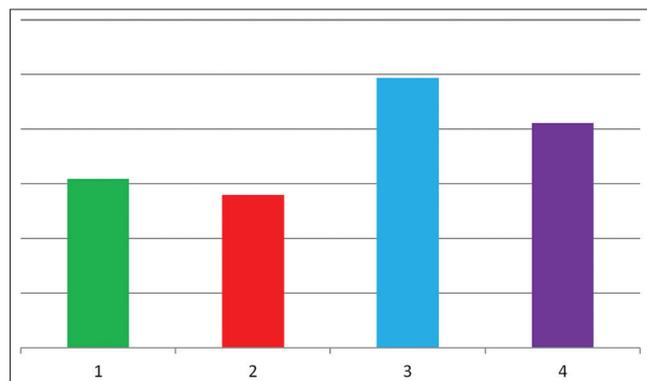


Fig. 2: Time taken for paralysis of the worms at highest concentration (75 mg/ml)

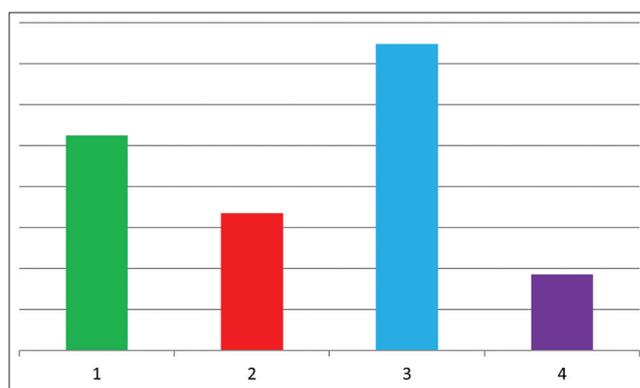


Fig. 3: Time taken for the death of the worms at highest concentration (75 mg/ml). (1) Chloroform leaf extract, (2) methanolic leaf extract, (3) aqueous leaf extract, (4) standard (piperazine citrate)

when shaken vigorously nor revive when dipped in normal saline. All the results were shown in Table 1.

## RESULTS

### Preliminary phytochemical screening

Preliminary phytochemical screening of the extracts revealed the presence of steroids, glycosides, flavonoids, and tannins.

### Anthelmintic activity

The crude extract of the leaves of *A. marina* produced a significant anthelmintic activity in a dose-dependent manner as shown in Table 1 and Fig. 1.

## DISCUSSIONS

From the observations made higher concentrations of extracts produced the paralytic effect much earlier and the time taken for death was shorter for all worms. The peak anthelmintic activity was exhibited by the methanolic extract at the highest concentration (75 mg/ml) which takes 13.98±0.94 min for paralysis and 33.49±0.69 min for the death of the worms, followed by the aqueous extract which takes 24.66±0.69 min for paralysis and 74.79±0.61 min for the death of the worms (Figs. 2 and 3). Chloroform extract exhibits less activity in comparison to other extracts. The leaves of *A. marina* shows promising anthelmintic activity as the other medicinal plants which are being explored for anthelmintic activity.

## CONCLUSION

The present study is designed to determine the effect of various extracts prepared from *A. marina* leaves. To the best of our knowledge, it is the first time that the leaves of *A. marina* were tested for anthelmintic

(1275/ac/09/CPSCEA) as per the guidelines given by CPCSEA on animal experimentation.

### Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.* [13,14]. The Indian earthworms were divided into three groups each containing six earthworms approximately equal size was selected for the experiment. A stock solution was prepared from the dried extract and different concentration (25, 50, and 75 mg/ml) were made from it, distilled water (control) and reference standard Piperazine citrate (25, 50, and 75 mg/ml in distilled water) taken for the study. The worms were then placed in Petri dish containing 25 ml of test sample and standard. The time taken to paralysis and death of the individual worms was recorded. The time taken to paralysis is noted when movement is observed only after vigorous shaking. Time taken for the death of worms was recorded after ascertaining that the worms neither moved

activity. In accordance with the results obtained it is concluded that the *A. marina* showed significant anthelmintic activity. The plant may be further explored to recognize the active chemical constituent responsible for the anthelmintic activity. If done it will be helpful to the natives living in tidal forests who cannot afford expensive synthetic anthelmintic drugs.

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#### AUTHOR'S CONTRIBUTION

I am solely responsible for the planning, conductance of study, interpretation of results and writing.

#### CONFLICT OF INTEREST

Do not have any conflicts of interest to include.

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