

# In vitro anthelmintic activity of *Acacia catechu* Willd

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**Aim:** The present study was designed to evaluate the anthelmintic activity of heartwood of *Acacia catechu* Willd. **Materials and Methods:** The alcoholic extract of heartwood of *Acacia catechu* Willd and its ethyl acetate fractions at different concentrations were tested on Indian adult earthworms (*Pheretima posthuma*) by *in vitro* standard procedure. Time of paralysis and time of death of the worms were considered as the parameters to assess the anthelmintic action. Albendazole and 2% w/v gum acacia in distilled water were used as standard and control respectively. **Results:** The ethyl acetate fraction of alcoholic extracts exhibited potent anthelmintic activity compared to alcoholic extract as evidenced by a significant decrease in time of paralysis and death. The observed activity could be due to the presence to phenolic compounds, particularly flavonoids in the test extract. **Conclusion:** These *in vitro* studies indicated that the *A. catechu* Willd. is a significant source of natural anthelmintic, which might be helpful in preventing the progress of various parasitic disorders.

**Key words:** *Acacia catechu*, alcoholic extract, anthelmintic activity, death, ethyl acetate fraction, paralysis

## INTRODUCTION

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause an enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia.<sup>[1]</sup> Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas.<sup>[2]</sup> Ideally an anthelmintic agent should have an broad spectrum of action, high percentage of cure with a single therapeutic dose, free from toxicity to the host and should be cost effective. None of the synthetic drugs available meets these requirements. Even most common drug like piperazine salts have been shown to have side effects like nausea, intestinal disturbances and giddiness.<sup>[3]</sup> Resistant of parasites to existing drugs and their high cost warrants the search for newer anthelmintic molecule. Hence, there is an increasing demand towards natural anthelmintics. The helmentic activity was evaluated on adult Indian earthworm, *Pheretima*

*postuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.<sup>[4,5]</sup>

*Acacia catechu* Willd. (Kattha, Khair, Kaath) belongs to family Mimosaceae. It has been traditionally used for the treatment of diarrhoea, dysentery, colitis, piles, ulcers, boils, skin eruptions, psoriasis, inflammation, leprosy, anaemia and leucoderma.<sup>[6]</sup> *A. catechu* Willd. contains tannins namely, catechutannic acid, acacia catechin, catechu red, catechin, epicatechin and flavonoids-quercetin and quercitrin.<sup>[7,8]</sup> *A. catechu* Willd. has been reported to possess various pharmacological activities like hypoglycaemic,<sup>[9]</sup> antibacterial,<sup>[10]</sup> antimycotic,<sup>[11]</sup> hepatoprotective,<sup>[12]</sup> hypotensive<sup>[13]</sup> and antiulcer<sup>[14]</sup> activity. Catechu, an active principle of *A. catechu* Willd. was reported to possess immunomodulatory activity.<sup>[15]</sup> Ethyl acetate extract of *A. catechu* Willd. showed antipyretic, antidiarrhoeal, hypoglycaemic and hepatoprotective activities.<sup>[16]</sup> Cyanidanol, an active principle of *A. catechu* Willd. is claimed to be effective in treating liver disorders.<sup>[17]</sup> The literature survey reveals that no reports were found proving anthelmintic activity of *A. catechu* Willd. Thus, this study was focused to investigate scientifically the anthelmintic activity of *A. catechu* Willd.

## MATERIALS AND METHODS

### Collection of Plant Material

The dried heart woods of *A. catechu* Willd. was procured from Yucca enterprises, Mumbai in the

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month of January 2012 and it was authenticated by Prof. B. Amarendhar Reddy, Sai Gouthami College, Ibrahimpatnam, R. R. District, Andhra Pradesh, India.

### Preparation of Extracts

The shade dried heart wood of *A. catechu* Willd. was coarsely powdered and extracted with absolute alcohol in Soxhlet apparatus for 72 h. The liquid extract was filtered and then concentrated using rotary flash evaporator at a temperature less than 45°C to get semisolid residue which was dried under vacuum. The dried extract was suspended in water and extracted with ethyl acetate, which was again concentrated and dried. The alcoholic extracts with its ethyl fractions were subjected for further studies.

### Preliminary Phytochemical Analysis

The preliminary phytochemical analysis was carried out to confirm presence of tannins and flavonoids.<sup>[18]</sup>

### Collection of Worms

Indian adult earthworms *Pheretima posthuma* were collected from Sri Krishna Vermiculture Pvt., Ltd. Uppariguda, Ibrahimpatnam, Hyderabad. The earthworms were identified by Prof. J. Srikanth, Department of Zoology, Sri Chaitanya Junior College, Hyderabad. The average size of earthworms being 6-8 cm. Prior to experiment, they were washed with tap water for the removal of the adhering dirt.

### Evaluation of Anthelmintic Activity

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. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug. The anthelmintic activity was carried as per method described by Panda *et al.*, with minor modifications. Both the test extracts and standard drug solution were freshly prepared before starting the experiment. The suspensions of test extracts were prepared in 2% gum acacia in distilled water to obtain dose of test drug at 25, 50, 75, 100 mg/ml. Albendazole suspension was also prepared in the same manner using 2% gum acacia in distilled water. The worms were divided into four groups each containing six worms. Grouping was done as follows:  
 Group I: Control (only the vehicle is used, i.e., 2% w/v gum acacia)  
 Group II: Alcoholic extract treated  
 Group III: Ethyl acetate fraction treated  
 Group IV: Standard drug (albendazole) treated.

Six worms were observed for their spontaneous motility and evoked responses. Time of paralysis is noted at different time intervals when no movement was observed except the worms were ascertaining that they neither moved even

when shaken vigorously, nor they revive even in normal saline. Time of death is noted when the worms showed zero response to the stimuli, even after performing the prick test and when dropped in warm water (50°C) followed with fading away of their body colour. All results were expressed as a mean  $\pm$  SEM of six animals in each group.<sup>[19-21]</sup> The observation of test extracts and standard drug is shown in the Table 1.

## RESULTS

From the observations made, a dose dependent paralytic effect much earlier and the time of death was observed [Table 1]. Although both the test extracts showed significant anthelmintic activity in a dose dependent manner, but the ethyl acetate fraction appeared to be more effective. Evaluation of anthelmintic activity was compared with reference standard albendazole. The alcoholic extract showed time of paralysis and time of death as 11.05 and 13.46 min. whereas for ethyl acetate fraction, it was 5.33 and 8.15 min. The reference standard albendazole showed the time of paralysis and time of death as 1.10 and 1.43 min, respectively.

## DISCUSSION

Preliminary phytochemical analysis of test extracts revealed the presence of tannins and flavonoids. Tannins have been reported to produce anthelmintic activities<sup>[22,23]</sup> as they can bind to free proteins in the gastrointestinal tract of host animal<sup>[24]</sup> or glycoprotein on the cuticle of the parasite and thereby cause deaths.<sup>[25]</sup> The potent wormicidal activity of ethyl acetate fractions against earthworms suggests that it is effective against parasitic infections of humans. As phytochemistry of *A. catechu* Willd. is well established, it can be concluded that phenolic compounds, specifically flavonoids (quercetin and quercetrin) are responsible for the potent wormicidal activity.

**Table 1: Anthelmintic activity of test extracts of *Acacia catechu* Willd**

Test drug	Conc. (mg/ml)	Paralysis time (min)	Death time (min)
Alcoholic extract	25	28.37 $\pm$ 0.06	45.13 $\pm$ 0.008
	50	25.30 $\pm$ 0.07	30.08 $\pm$ 0.05
	75	20.23 $\pm$ 0.07	26.58 $\pm$ 0.06
	100	11.05 $\pm$ 0.06	13.46 $\pm$ 0.004
Ethyl acetate extract	25	10.28 $\pm$ 0.06	014.60 $\pm$ 0.03
	50	08.42 $\pm$ 0.06	010.53 $\pm$ 0.05
	75	07.12 $\pm$ 0.06	09.53 $\pm$ 0.08
	100	05.33 $\pm$ 0.77	08.15 $\pm$ 0.006
Albendazole	25	03.38 $\pm$ 0.04	04.17 $\pm$ 0.006
	50	01.53 $\pm$ 0.008	04.15 $\pm$ 0.012
	75	01.22 $\pm$ 0.007	02.14 $\pm$ 0.003
	100	01.10 $\pm$ 0.007	01.43 $\pm$ 0.0080

Result expresses as mean $\pm$ SEM from six observations

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