In vitro Anthelmintic Activity of Leaf Extracts of *Adhatoda vasica N*ees (Acanthaceae) *A*gainst *Eudrilus eugeniae*

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ABSTRACT: The present study specifically indicated that the crude ethanolic and aqueous extracts of the leaves of *Adhatoda vasica Nees* produced anthelmintic activity against african earthworm *Eudrilus eugeniae*. Various concentrations (10, 25, 50 mg/ml) of aqueous and ethanolic extracts were evaluated in the bioassay involving determination of time of paralysis (P) and time of death (D) of the worms. Albendazole was used as standard anthelmintic drug and distilled water was used as negative control. The results of the present study indicated that the ethanolic and aqueous extracts significantly exhibited paralysis of worms in lower doses (10, 25 and 50 mg/ml) and also caused death of worms at higher concentration of 50 mg/ml, as compared to standard drug. Further studies are in process to isolate the active principle responsible for the activity.

Key words: Albendazole, anthelmintic activity, *Adhatoda vasica*, *Eudrilus eugeniae*

INTRODUCTION

Helminthes infections or helminthiasis are among the most the pervasive infection. It is degenerative disease distressing a large proportion of world's population. In developing countries, they pose a serious threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia.[1] Highly effective and selective anthelmintic drugs are available, but such compounds must be used correctly, judiciously, and with consideration of the parasite/host interaction to obtain a favorable clinical response, accomplish good control, and minimize selection for anthelmintic resistance. So the research for new agents is continuing.

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Leaves of *Adhatoda vasica nees* is a shrub native to Asia, commonly known as Malabar nut, which grows also in Sri Lanka, Nepal, India and the Pothohar region of Pakistan and in the Pharwala area.[2] It has multiple traditional uses in folk medicine. Mostly it is used in the treatment of asthma and cough.[3] Additionally, they are used in cold, cough, whooping cough, chronic bronchitis and asthma as sedative expectorant, antispasmodic and anthelmintic.[4] Al-Shaibani *et al.*[5] have studied the anthelmintic activity of aerial parts of *A. vasica* against gastrointestinal nematodes of sheep but we have investigated anthelmintic activity by taking leaves of *A. vasica* only. The method reported by Mali *et al.*[6] was excluding the use of Dunnets't' test, which was found to be the limitation of the method. Also they used *Pheretima posthuma* (indian adult earthworm) but we have studied on *E. eugeniae* earthwarm (African species) and Dunnets't' test which are novelty of our research work. The assay was performed on adult African species of earthworm *E. eugeniae*.[7] These are easily available and used as a

suitable model for screening of anthelmintic drug.[8-10] In the last few years, there has been an exponential growth in the field of herbal medicine, which is gaining popularity in both developing and developed countries because of their natural origin and less side effects.[11] It is important to know the active components and their molecular interactions, which will help to analyze the therapeutic efficacy of the future perspective drugs. Therefore, we studied the anthelmintic activity of leaf extract of *A. vasica.*

The leaves of *A. vasica* were collected from Atpadi, Sangli, Maharashtra, India in the month of August, 2013. The plant was identified by local people of the village and authenticated by Dr. G. G. Potdar, Professor, Y. C. College of Pharmacy, Karad, Satara, Maharastra, India and the voucher specimen is preserved in the laboratory for future reference. All the reagents used were of analytical grade procured from S.D Fine Chemicals Ltd., and Hi Media, Mumbai.

Leaves of *A. vasica* were dried under shade, powdered and stored in closed vessel for further use. The dried powder material (50 g) was subjected to soxhlet extraction with ethanol and water for 6 h. The extracts were concentrated under reduced pressure to obtain solid residues. The percentage value of the ethanolic and aqueous extracts was 9.40 % w/w and 10.20 % w/w, respectiviely.

All the experiments were carried out in African adult earth worm *E. eugeniae* (Annelida) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil of Kasegaon Agriculture Field, Kasegaon, Tal-Walwa, Sangli, Maharashtra (India) and washed with water to remove all fecal matters.

Samples for *in vitro* study were prepared by dissolving and suspending 2.5 g of each extract (ethanolic and aqueous separately) 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 final concentration of 10, 25 and 50 mg/ml.

Table 1. Anthelmintic activity of leaves extract of the *A. vasica*.

Test subject	Concentration mg/ml	Time taken for paralysis (P) and death of worms (D) in min	
		P	D
Vehicle	-	-	-
Albendazole	25	9.4001 ± 0.1028	14.4391 ± 0.1509
Aqueous extract	10	17.2655 ± 0.1451**	40.5255 ± 0.1132**
	25	12.5338 ± 0.1564**	30.5353 ± 0.1469**
	50	9.4806 ± 0.1016**	22.4210 ± 0.1313*
Ethanolic extract	10	11.4341 ± 0.1317**	20.3228 ± 0.1021**
	25	8.1112 ± 0.0896**	16.05230 ± 0.1494**
	50	4.5011 ± 0.1457**	10.4350 ± 0.1434**

Values are expressed as Mean±SEM
One way ANOVA followed by Dunnets't' test.
Note: n=6 in each group. **Significant, *Non significant

The anthelmintic assay was carried out as per the previous method with minor modifications.[12-15] The 50 ml formulations containing different concentrations of each ethanolic and aqueous extract (10, 25, and 50 mg/ml in distilled water) were prepared and twelve worms (same size) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when 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 ºC.[16,17] Albendazole (25 mg/ml) was used as reference standard while distilled water as the negative control.

As shown in table 1, the aqueous and ethanolic extracts of *A. vasica* exhibited anthelmintic activity

on *E. eugeniae* worms in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration shown in figures 1 and 2. The ethanolic extract caused paralysis at 4.5011 ± 0.1457 min. and time of death was 10.4350 ± 0.1434 min while the aqueous extract revealed paralysis in 9.4806 ± 0.1016 min and time of death of 22.4210 ± 0.1313 min, respectively against the earthworm *E. eugeniae*. The standard drug albendazole showed the paralysis at 9.4001 ± 0.1028 min and time of death of 14.4391 ± 0.1509 min for 25 mg/ml.

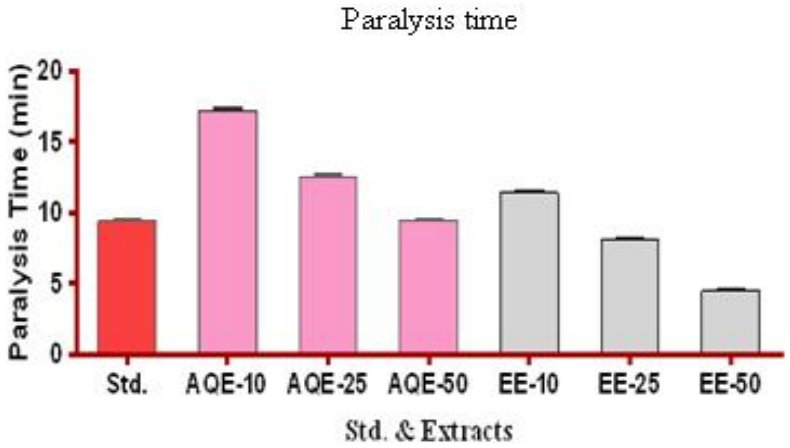

Figure 1. Paralysis time of *A.vasica* leaves against *E. eugeniae.*

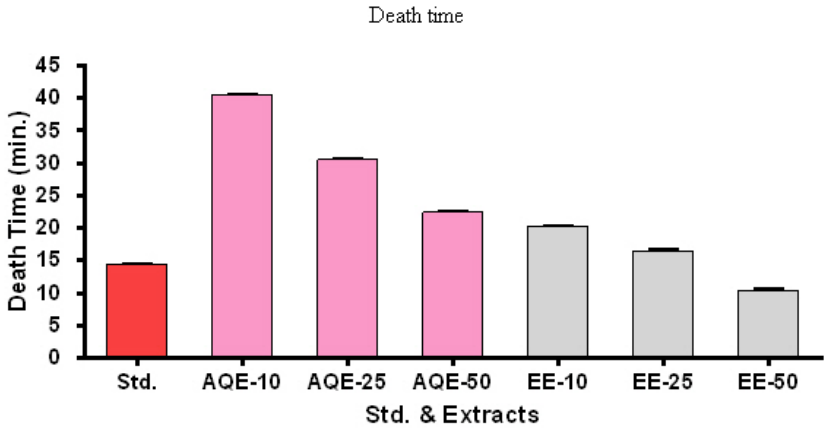

Figure 2. Death time of *A.vasica* leaves against *E. eugeniae.*

Albendazole kills the worms by increasing chloride ion conductance in worm muscle membrane whice produces hyperpolarization and reduces excitability that led to muscle relaxation and flaccid paralysis.[18] Ethanolic and aqueous extracts of *A. vasica* 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 albendazole. Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents. Tannins were shown to produce anthelmintic activities. Chemically tannins are polyphenolic compounds. It is possible that tannins contained in the whole extract of *A. vasica* produce similar effects. Reported anthelmintic effect of tannins is caused by their binding to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death.[19,20]

The study has shown that ethanolic and aqueous leaves extracts of *A. vasica* have significant anthelmintic activity. But the ethanolic extract of *A. vasica* showed most significant anthelmintic activity as compared to the aqueous extracts and standard. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

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