In vitro Anthelmintic Activity of Leaf Extracts of Adhatoda vasica Nees (Acanthaceae) Against 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. 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.

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. It has multiple traditional uses in folk medicine. Mostly it is used in the treatment of asthma and cough. Additionally, they are used in cold, cough, whooping cough, chronic bronchitis and asthma as sedative expectorant, antispasmodic and anthelmintic. Al-Shaibani et al. 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. 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 earthworm (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. 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, Maharashtra, 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, respectively.

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>
<thead>
<tr>
<th>Table 1. Anthelmintic activity of leaves extract of the A. vasica.</th>
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<td>Test subject</td>
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<tr>
<td>Vehicle</td>
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<td>Albendazole</td>
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<td>Aqueous extract</td>
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Values are expressed as Mean±SEM
One way ANOVA followed by Dunnet’s 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 °C.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.

![Paralysis time](image1.png)

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

![Death time](image2.png)

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

Albendazole kills the worms by increasing chloride ion conductance in worm muscle membrane which produces hyperpolarization and reduces excitability that led to muscle relaxation and flaccid paralysis. 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.
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 phytocomponents responsible for anthelmintic activity.

REFERENCES


