Anthelmintic activity of Alocasia indica Schott. rootstocks.

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Abstract
The present study was designed to evaluate the anthelmintic activity of Alocasia indica Schott. rootstocks using specific in vitro standard procedures. The alcoholic extract of rootstocks of Alocasia indica Schott. & its ethyl acetate fraction at different concentrations were tested on Indian adult earthworms (Pheretima posthuma). Time of paralysis and time of death of earthworms 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. The ethyl acetate fraction of alcoholic extract exhibited significant anthelmintic activity compared to alcoholic extract as evidenced by significant decrease in time of paralysis & death. The observed activity could be due to the presence to phenolic compounds, particularly flavonoids in the test extract. These in vitro studies indicated that the Alocasia indica Schott. is a significant source of natural anthelmintic, which might be helpful in preventing various parasitic disorders.

Key words:
Alcoholic extract, Alocasia indica Schott., anthelmintic activity, death, ethyl acetate fraction, paralysis

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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 enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia \[1\]. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas \[2\]. 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 & 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 & giddiness \[3\]. Resistant of parasites to existing drugs & 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 posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.\[4,5\]

*Alocasia indica* Schott. is a perennial herb with stout rootstock, found tropical and sub tropical regions of India. The *Alocasia indica* Schott. (Family- Araceae) commonly known as Giant Taro is a perennial herb found throughout greater part of India. \[6\] According to ayurvedic literature survey, different parts of this plant are traditionally used as hepatoprotective, antioxidant, analgesic, antiarthritic, anti-inflammatory, antitumour & antipyretic. It is also reported to use in the treatment of piles.\[7\] Alcoholic extract of leaves were evaluated for antimicrobial\[8\], antidiarrhoal,\[9\] antioxidant\[10\], anti-inflammatory\[10\], & anthelmintic\[11\] properties. Seeds extract is reported for its antifungal activity.\[12\] Literature survey revealed that the plant contains flavonoids, alkaloids, cyanogenetic glycosides, steroids, gallic acid, succinic acid, ascorbic acid, amino acids, oxalic acid,\[7\] & alocasin.\[13\] It is evident that the plant has great potentials in treating various diseases. Thus, the present investigation was aimed to evaluate the anthelmintic activity of *Alocasia indica* Schott.

**MATERIALS & METHODS**

**Collection of plant material**

*Alocasia indica* Schott. root stocks were collected in the month of January 2011, from local areas of Ibrahimpatnam, Hyderabad, (A.P) and authenticated by Prof. B. Amarendhar Reddy, Sai Gouthami College, Ibrahimpatnam, R.R.Dist, A.P, India.

**Extraction**

The shade dried rootstocks of *Alocasia indica* Schott. was coarsely powdered & extracted with absolute alcohol in soxhlet apparatus for 72 hrs. The liquid extract was filtered & then concentrated using rotary flash evaporator at a temperature less than 45°C to get semisolid residue which was dried under vaccum. The dried extract was suspended in water and extracted with ethyl acetate which was again concentrated and dried. The alcoholic extract with its ethyl fractions were subjected for further studies.

**Preliminary phytochemical screening**

Total alcoholic extract and its ethyl acetate fractions of rootstocks of *Alocasia indica* Schott. were subjected for qualitative chemical analysis.\[14\]

**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, Dept. 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.
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 Mali *et al* with minor modifications. Both the test extracts & 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 6 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 ± SEM of six animals in each group.[15-17]

**Results & Discussion**

From the observations made, a dose dependent paralytic effect much earlier and the time of death was observed (Table no. 1). Although both the test extracts showed 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 & time of death as 41.10 & 43.51 min. respectively whereas for ethyl acetate fraction it was 5.33 & 8.15 min respectively. The reference standard albendazole showed the time of paralysis & time of death as 1.10 & 1.43 min. respectively.

Preliminary phytochemical analysis of test extracts revealed the presence of steroids & phenolic compounds (flavonoids). Flavonoids have been reported to produce anthelmintic activities[18,19] as they can bind to free proteins in the gastrointestinal tract of host gastrointestinal tract of host animal[20] or glycoprotein on the cuticle of the parasite[21] and cause death.

**Conclusion**

The ethyl acetate fraction of alcoholic extract of *Alocasia indica* Schott. rootstocks exhibited significant anthelmintic activity against earthworms in dose dependent manner. The observed activity may be due to their phenolic content (flavonoids) which is worth for further investigations on isolation of the specific constituents.

**Table 1:** Anthelmintic Activity Of Test Extracts Of *A. indica* Schott. Rootstocks

<table>
<thead>
<tr>
<th>Test Drug</th>
<th>Conc. (mg/ml)</th>
<th>Paralysis Time (min.)</th>
<th>Death Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic Extract (AE)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>25</td>
<td>55.42 ± 0.06</td>
<td>75.18 ± 0.008</td>
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</tr>
<tr>
<td>50</td>
<td>55.35 ± 0.07</td>
<td>60.13 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>50.28 ± 0.07</td>
<td>36.63 ± 0.06</td>
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</tr>
<tr>
<td>100</td>
<td>41.10 ± 0.06</td>
<td>43.51 ± 0.004</td>
<td></td>
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<tr>
<td>Ethyl Acetate Fraction (EAF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>10.28 ± 0.06</td>
<td>14.60 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>08.42 ± 0.06</td>
<td>10.53 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>07.12 ± 0.06</td>
<td>09.53 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>05.33 ± 0.77</td>
<td>08.15 ± 0.06</td>
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<tr>
<td>Albendazole (S)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>03.38 ± 0.04</td>
<td>04.17 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>01.53 ± 0.008</td>
<td>04.15 ± 0.012</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>01.22 ± 0.007</td>
<td>02.14 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>01.10 ± 0.007</td>
<td>01.43 ± 0.008</td>
<td></td>
</tr>
</tbody>
</table>
Result expresses as mean ± SEM from six observations.

Fig. 1: Histogram showing anthelmintic activity of test extracts of A. indica Schott rootstocks.

References