IN VITRO ANTHELMINTIC ACTIVITY OF CASEARIA VARECA ROXB.-AN ENDEMIC PLANT OF ASSAM

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ABSTRACT
Ethyl acetate and Methanolic extracts from the leaves of Casearia vareca Roxb. (Flacourtiaceae) were investigated for their anthelmintic activity against adult Indian earthworm, Pheretima posthuma and nematode, Ascardia galli. Various concentrations (10, 20, 50 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Both the extracts exhibited significant anthelmintic activity at the highest concentration of 50 mg/ml. Piperazine citrate (15 mg/ml) and Albendazole (20 mg/ml) were used as standard references while 0.5% carboxy methyl cellulose (CMC) in normal saline as control.

KEYWORDS: Casearia vareca, Anthelmintic activity, Pheretima posthuma, Ascardia galli.

INTRODUCTION
Casearia vareca Roxb. (Flacourtiaceae) is a popular and socio-culturally recognized plant in Assamese community and is commonly known as Chhagladoi or Sikrai. The young leaves and shoots are edible and cooked as vegetable by Assamese people (Borthakur, 1996). The fruits are rubbed into a paste and given to people suffering from worms, while the juice of the fruits is dropped into the ear when attacked by ticks (Kanjilal, 1997). The plant is mentioned in the Dictionary of Indian Folk Medicine and Ethnobotany and use for worm infection, earache, fever, headache, vermifuge and as anticancer (Jain, 1991). Assamese people use this plant leaves traditionally to treat various diseases, like injuries, burns, abscess, pain, and in worm infection. The plant is also considered to be effective in the treatment of diarrhoea and...
dysentery. The plant is distributed all over the North East and up to 3,000 ft., in the Hills. A botanical description of the plant is recorded in Flora of Assam (Kanjilal, 1997).

MATERIALS AND METHODS
Plant material *Casearia vareca* Roxb. (Flacourtiaceae) was collected in the month of June from Naharkatia, Dibrugarh dist. Assam, Assam, India. The taxonomical identification and authentification of the plant was done by the Botanical Survey of India, eastern circle, Shillong, Meghalaya. The identification report No. BSI/EC/Identification/2009/289, dated: 20/07/2009) was preserved in Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India for future reference.

Preparation of the extract
The collected plant leaves were cleaned, shade dried, coarsely powdered. The powder was defatted with petroleum ether (60-80°C). It was then successively extracted in a Soxhlet apparatus using ethyl acetate and methanol as solvent. The solvent was allowed to evaporate in a rotary vacuum evaporator. The vacuum dried extracts obtained were subjected to preliminary screening of phytochemicals (Trease, 1989) and anthelmintic activity.

Drug and chemicals used
Piperazine citrate and Albendazole (Glaxo Smith Kline Pharmaceuticals Ltd., Mumbai) were used as reference standards. Chemicals: Petroleum ether AR, ethyl acetate AR (RFCL Ltd., New Delhi), ethanol (99.9%) (Hong Yang Chemical Corporation, China) and carboxy methyl cellulose (CMC).

Animals
*Pheretima posthuma* (Annelida), commonly known as Indian earth worm were collected from the water logged area of Dibrugarh and *Ascardia galli* (nematode) worm were obtained from freshly slaughtered fowls (*Gallus gallus*). Both the worm types were identified at the department of Zoology, DHSK College, Dibrugarh, Assam and department of Parasitology, College of Veterinary Science, Khanapara, Guwahati, Assam, respectively.

Evaluation of anthelmintic activity
The anthelmintic activity was carried out as per the method of Ajaiyeoba et al., 2001. The activity was evaluated on adult Indian earth worm *Pheretima posthuma* (Shivkar and Kumar, 2003; Sollmann, 1918) as well as on worm parasites of human beings *Ascardia galli*
(Nematode) which are available in slaughtered fowls (Deore et al., 2009; Kaushik et al., 1974). Nine groups of worms were used to assess the anthelmintic properties of the extracts of *Casearia vareca*. Group 1 were the control worms placed in 0.5% CMC in normal saline; groups 2-4 treated with 10, 20 and 50 mg/mL of ethyl acetate leaves extract in 0.5% CMC in normal saline; groups 5-7 treated with 10, 20 and 50 mg/mL of methanolic leaves extract in 0.5% CMC in normal saline; group 8 with Piperazine citrate in normal saline; and group 9 with Albendazole in normal saline. Each group included six worms of each type. Observations were made for the time taken to set paralysis and death of individual worms. Mean time for paralysis (P) in min. was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The time for death (D) in min. was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Piperazine citrate (15 mg/mL) (Mali, et al., 2004) and Albendazole (20 mg/kg) (Kumar et al., 2010) were used as reference standards while 0.5% CMC in normal saline as control.

RESULTS

The results of the preliminary phytochemical screening are shown in Table 1. From the anthelmintic assay, it was observed that the ethyl acetate and methanolic leaves extracts of *Casearia vareca* not only produced paralysis but also caused death of both species of worms. As shown in the Table 2, both the extracts exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) as well as death (D) with 50 mg/mL concentration, for both types of worms. The anthelmintic activity were also observed for the tested standards (i.e., Piperazine citrate and Albendazole), although Piperazine citrate caused only paralysis, not death to the worms. Furthermore, the anthelmintic activity of the extracts of *Casearia vareca* was comparable with the standard drugs, while it caused both paralysis and death of the worms similar to Albendazole.

Table 1. Preliminary phytochemical tests of bark extracts of *Casearia vareca* Roxb.

<table>
<thead>
<tr>
<th>Plant Constituents</th>
<th>Ethyl acetate extract</th>
<th>Methanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fats &amp; Oils</td>
<td>_</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gums</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Steroids</td>
<td>_</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>_</td>
<td>+</td>
</tr>
</tbody>
</table>
Saponins | + | +  
Tannins | + | +  
Lignin | - | +  
Triterpenoids | - | -  

(+) means present and (-) means absent.

Table 2. Anthelmintic activity of ethyl acetate and methanolic extracts of *Casearia vareca* Roxb.

<table>
<thead>
<tr>
<th>Test</th>
<th>Concentration (mg/ml)</th>
<th>Time taken for paralysis (P) and death (D) of worms(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>P. posthuma</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paralysis time</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate extract(CV-1)</td>
<td>10</td>
<td>62.02±0.31</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>17.05±0.30</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.12±0.25</td>
</tr>
<tr>
<td>Methanolic extract(CV-2)</td>
<td>10</td>
<td>63.46±0.33</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>18.68±0.32</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6.54±0.32</td>
</tr>
<tr>
<td>Piprazine citrate</td>
<td>15</td>
<td>18.26±0.37</td>
</tr>
<tr>
<td>Albendazole</td>
<td>20</td>
<td>35.31±0.33</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SEM from six observations.

**Fig. 1.** Anthelmintic activity of ethyl acetate and methanolic extracts of *Casearia vareca* Roxb.

**DISCUSSION**

Anthelmintic activity was performed on adult Indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Chatterjee, 1967; Thorn, *et al.*, 1977). Because of easy availability,
earthworms have been used widely for the initial evaluation of anthelmintic activity of compounds in-vitro (Dash et al., 2002; Jain et al., 1972; Szewezuk, et al., 2003). *Ascardia galli* (Nematode) are available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic compounds was advocated (Lal et al., 1976; Tandon et al., 1997). The predominant effect of Piperazine citrate on the worms is to cause a flaccid paralysis that result in expulsion of the worms by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis (Martin, 1985). On the other hand, Albendazole causes death of the worm. The lethal effect of Albendazole is attributed to its inhibition of β- tubulin polymerization and thus interfering with microtubule-dependent glucose uptake by the worms (Tripathi, 2008; Rang, et al., 2005). The leaves extracts of *Casearia vareca* were not only demonstrated paralysis, but also caused death of both worms, which could be attributed to its inhibition of tubulin polymerization and blocking glucose uptake due to its similarity in action with Albendazole. Furthermore, the anthelmintic activity may be attributed to the phytoconstituents present in the plant, jointly or separately. In conclusion, the traditional claim of the leaves of *Casearia vareca* as an anthelmintic have been confirmed as the leaves extracts displayed activity against the worms used in the study. The study justifies its use in curing helmintic infestation and may provide some pharmacological rationale for the folklore use. Further studies to isolate and reveal the active component(s) contained in the crude extracts of the leaves of *Casearia vareca* and to establish the mechanism of action are required.

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REFERENCES


