In vitro Licicidal Activity Of Different Extracts Of Acorus calamus Linn. (Araceae) Rhizome.

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Abstract
Acorus calamus L. is well known for Insecticidal and Pesticidal activity. In present study dried rhizomes of A. calamus were subjected to exhaustive sequential extraction with four solvents n-hexane, chloroform, methanol and distilled water respectively. All four fractions were studied for in vitro licicidal activity using Goat-lice Damalinia caprae (Trichodectidae) as experimental organism. Only n-hexane and chloroform fractions showed licicidal activity. Statistically significant (p value < 0.01, n = 5) decrease in mean time required to kill the lice was observed at concentration 1% w/w and 10% w/w when compared to 1% w/w lindane solution.

Keywords: Acorus calamus, rhizome, Licidal activity.

Introduction and Experimental
Acorus calamus L. (Araceae) commonly known as “Bach or Vacha” is a semiaquatic herb with creeping rhizomes and sword shaped long leaves found throughout India near marshy places, river banks and lakes1. All parts of the plant contain volatile oil. The volatile oil contain terpenoids calamine, calamenol, calamenone, eugenol, camphene, pinene and asaronaldehyde. Acorafuran is a new sesquiterpenoid found in Calamus oil2.

Various pharmacological activities have been reported with A. calamus3-11. The aim of present work was to evaluate licidial activity of different fractions obtained by exhaustive sequential extraction of A. calamus rhizomes by using in vitro method.

Feeding habit and anatomy of Goat lice Damalinia caprae12 closely resemble human Head-lice Pediculus humanus (capitis) hence Goat lice was used as experimental organism. 1% lindane topical lotion is commonly used synthetic insecticide to treat lice infestation hence licidal activity of A. calamus was compared with lindane13.
Plant Material
Rhizomes of *Acorus calamus* Linn. (Araceae) were collected from Darwha District-Yavatmal (MS). These were identified and authenticated by Dr. Mrs. Prabha Bhogaoankar, Director Govt. Vidarbha Institute of Science and Humanities, Amravati. The rhizomes were dried under shade for 15 days and ground to coarse particle size.

Extraction
100 Gm. coarse powder of *A. calamus* rhizome was exhaustively extracted in Soxhelet apparatus successively by using n-hexane, chloroform and methanol. Each time marc was dried at 45°C and solvent was evaporated at 45°C to get respective fraction. Finally the dried marc was boiled with distilled water for 30 minutes, filtered and filtrate was evaporated at 45°C to get dry aqueous fraction.

Test Solutions
0.1, 1, 10 % w/w Test solutions of n-Hexane fraction and Chloroform fraction were separately prepared in coconut oil.
0.1, 1, 10 % w/w Test solutions of Methanol fraction and Aqueous fraction were separately prepared in Distilled water.

Standard Solution
1 % w/w lindane solution.

Experimental Organism
Goat-lice *Damalinia caprae* (Trichodectidae) were collected from healthy goat located in Amravati (MS).

In an earlier observation independent of present study the lice were found to remain live for 24-48 hours when removed and kept away from host body.

Experimental Procedure
This was carried out by modifying a method used by Pollack *et al.* (1999)\(^1^4\). A 25 Ml. capacity glass beaker was taken. A filter paper disc coinciding with internal diameter of the beaker was cut. 0.15 Gm. test solution was applied as thin layer on bottom of beaker and on the disc by using brush, then the disc was placed in beaker. A group of 5 lice was placed over the disc and observed through magnifying glass.

The time in minutes at which each louse became immobile was noted down.
The immobilized lice were taken out, placed on fresh dry filter paper and observed for 6 hours at interval of 30 minutes. The lice which did not show movement during this period were considered dead at the time when these were became immobile. Same procedure was carried out for each test solution and standard solution.

Statistical Analysis
Separate group of 5 lice was assigned to each test solution and standard drug hence n = 5. All the values expressed as Mean ± S.E.M. The data was analysed by Student’s t test. p value < 0.01 was considered statistically significant.
Table 1: Effect of *A. calamus* on Goat-lice *Damalinia caprae*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean Time in Minutes ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.1 % w/w n-Hexane fraction test solution</td>
<td>156 * ± 1.87</td>
</tr>
<tr>
<td>Group 2</td>
<td>1 % w/w n-Hexane fraction test solution</td>
<td>67 * ± 3.00</td>
</tr>
<tr>
<td>Group 3</td>
<td>10 % w/w n-Hexane fraction test solution</td>
<td>33 * ± 3.00</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.1 % w/w Chloroform fraction test solution</td>
<td>168 * ± 2.55</td>
</tr>
<tr>
<td>Group 5</td>
<td>1 % w/w Chloroform fraction test solution</td>
<td>84 * ± 1.87</td>
</tr>
<tr>
<td>Group 6</td>
<td>10 % w/w Chloroform fraction test solution</td>
<td>45 * ± 2.24</td>
</tr>
<tr>
<td>Group 7</td>
<td>1 % w/w Lindane solution (standard)</td>
<td>95 ± 5.25</td>
</tr>
</tbody>
</table>

*n = 5 in each group. Values are Mean time in minutes at which lice were considered dead ± S.E.M. *p value < 0.01

Results and Discussion

Methanol and Aqueous fractions not showed licicidal activity. Values of Mean time in minutes at which lice became immobile and considered dead on treatment with n-Hexane fraction, Chloroform fraction and Standard drug are given in Table 1. Both n-Hexane and Chloroform fractions showed significant decrease in the Mean time required to kill lice with 1% and 10% concentrations where as increase in the Mean time was observed with 0.1% concentration when compared to 1 % lindane. n-Hexane fraction showed more potent activity than Chloroform fraction at all concentrations 0.1, 1 and 10% w/w.

Thus from the present study it is evident that *A. calamus* L. rhizomes contain chemical constituents responsible for licidal property and the constituents are predominately soluble in n-Hexane.
References