In vitro filaricidal activity of some folklore medicinal plants of Manipur, India

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Diethylcarbamazine (DEC), a drug in long use since 1947, is a good microfilaricide but produces serious side reactions (Rollo, 1970; Partono et al., 1979). The WHO Special Programme on tropical diseases has given highest priority to the development of new antifilarial drugs, particularly, the ones that could kill the adult worms (WHO, 1991). The recent human trials of Ivermectin, a newly synthesized filaricidal drug, showed that though it reduces the microfilarial concentration in bancroftian filariasis, yet a recurrence of microfilaraemia was noticed after its treatment (Cartel et al., 1990). In the search for a suitable antifilarial drug, several plants or their compounds have been tested for their putative efficacy against filariid worms (Comley, 1990). The state of Manipur (India) is richly inhabited by various tribes, who in their traditional medical system, use a number of plants or plant-derived preparations for curing several ailments, including filariasis. Primarily, with a view to evaluate the filaricidal potentials of these plants, the present study was undertaken to examine the efficacy of methanolic extracts of these plants against filarial worms of Setaria cervi, in vitro.

MATERIALS AND METHODS

Preparation of plant extracts:

The collected plant materials (Table-1) were air-dried under shade and later ground into powder for their extraction using methanol as an organic solvent, by employing the Soxhlet Fractional Distillation method (Gafner et al., 1985). The final recovery of extracts was done using a Rotatory Evaporator. The details regarding the amount of fresh plant

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materials, weight after drying and weight of the final recovery of the crude extracts in each case are provided in Table 2.

**Experimental design:**

Adult *S. cervi* were collected in warm saline from freshly slaughtered cattle at local abattoirs, Mawlai, Shillong. They were maintained in pairs in Hank’s solution at 37±2 °C temperature in an incubator. The plant extracts were dissolved in a few drops of Dimethyl Sulphoxide (DMSO) and tested at concentrations of 5, 10, 20 and 40 mg/ml in each case. Corresponding concentrations of diethylcarbamazine (DEC), a standard antifilarial drug was also tested to compare the efficacy of individual plant extracts. In each case, a set of worms maintained without plant extract but having a few drops of DMSO in the petridish served as controls.

The antifilarial efficacy of plant extracts was adjudged in terms of the motility and mortality of the test parasites and was monitored at every half an hour time-interval. In order to confirm the motility and mortality of parasites, two grades (one warm and the

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Scientific name</th>
<th>Local name</th>
<th>Place of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Artemisia ambrosifolia</em></td>
<td>Maharni</td>
<td>Peh</td>
</tr>
<tr>
<td>2.</td>
<td><em>Trifolium repens</em></td>
<td>Anikatum</td>
<td>Paoyi</td>
</tr>
<tr>
<td>3.</td>
<td><em>Xanthoxylum rhetsa</em></td>
<td>Mangangteini</td>
<td>Mungreitang</td>
</tr>
<tr>
<td>4.</td>
<td><em>Solanum myriscanthum</em></td>
<td>Changranlotei</td>
<td>Shampirum</td>
</tr>
<tr>
<td>5.</td>
<td><em>Carica papaya</em></td>
<td>Awathabi</td>
<td>Paoyi Atotang</td>
</tr>
</tbody>
</table>

**Table 2. Plant extractions and recovery of final extracts**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant</th>
<th>Fresh material (g)</th>
<th>Dried material (g)</th>
<th>Ground Powder (g)</th>
<th>Extract (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>A. ambrosifolia</em></td>
<td>1200</td>
<td>345</td>
<td>200</td>
<td>48.35</td>
</tr>
<tr>
<td>2.</td>
<td><em>T. repens</em></td>
<td>1050</td>
<td>90</td>
<td>90</td>
<td>15.82</td>
</tr>
<tr>
<td>3.</td>
<td><em>X. rhetsa</em></td>
<td>890</td>
<td>125</td>
<td>120</td>
<td>17.83</td>
</tr>
<tr>
<td>4.</td>
<td><em>S. myriscanthum</em></td>
<td>1090</td>
<td>280</td>
<td>200</td>
<td>37.82</td>
</tr>
<tr>
<td>5.</td>
<td><em>C. papaya</em></td>
<td>2200</td>
<td>120</td>
<td>120</td>
<td>29.82</td>
</tr>
</tbody>
</table>
RESULTS

The mean mortality time of *S. cervi* maintained in the control condition was recorded to be 10.75 hours, whereas, the mortality range varied from 10.00 to 13.00 hours. With regard to DEC, the mean mortality time recorded for 5 and 10 mg/ml concentrations was 0.67 hours, whereas the same was found to be from 0.50 hours for both 20 and 40 mg/ml concentrations. The range of mortality in this case was tabulated to be from 0.50 to 0.67 hours.

The mean mortality time of various plant extracts tested at maximum concentrations of 40 mg/ml varied from a minimum of 0.67 hours in case of *T. repens*, *S. myriscanthum* and *C. papaya*, whereas, the same was recorded to be 0.83 and 1.00 hours in case of *A. ambrosifolia* and *X. rhetsa*, respectively. The range of mortality for individual plant extracts did not show much variation as it varied between a minimum of 0.67 hours to a maximum of 2.83 hours (Table 3).

DISCUSSION

The present study suggests that the effects of plant extracts on *S. cervi* were concentration dependent. An early mortality of parasites was observed at relatively high (40 mg/ml) concentration of each plant extract. In view of this, it may be assumed that the macrofilaricidal ingredients may be present in the crude extracts of these plants.

Several other medicinal plants tested for their antifilarial efficacy have also shown similar kind of concentration dependent results (Comley, 1990). Khunkitti *et al.*, (2000) in their *in vitro* antifilarial study of a plant, *Cardiospermum halicacabum* against *Brugia pahangi*, reported that adult worms cultured for 7 days in ≥ 0.5 mg/ml its aqueous extract showed complete damage. The aqueous extract, however, was found to be less effective on microfilariae. Conversely, the ethanol extract of the same plant was effective against microfilariae, but not against the adult worms. This pattern of stage-dependent activity was also found in case of treatment with the extracts of plants *Streblus asper* and *Sencio nudicaulis* against *S. cervi*, *in vitro*, by Parveen *et al.* (1989) and Singh *et al.* (1996). In another related study, the extracts of Solamargine, isolated from *Solanum khasianum* killed 100% adults of *Setaria cervi*, *in vitro*, at 4 mg/ml concentration in 60 minutes and the extract of the two triterpenoid saponins isolated from *Acacia auriculiformis* killed 100% adults of *S. cervi* *in vitro* in 35 minutes at the same concentration (Ghosh *et al.*, 1993, 1994). An *in vitro* treatment of DEC on adult *S. cervi* also resulted into 100% mortality at the concentration of 750 µg/ml in 24 hours (Singhal *et al.*, 1973).
The present study, therefore, substantiates the use of these plants as folklore medicines. Further studies need to be carried out to define the constituent agents responsible for these potential effects.

SUMMARY

People, in their indigenous medicinal system, employ several plants that are believed by them to be vermicidal without any scientific basis to explain their efficacy or action. The present study reports the filaricidal efficacy of some plants that are used in the folklore medicinal system of native tribes of Manipur, India using *Setaria cervi* (a bovine filariid) as a test parasite. The efficacy was monitored in terms of motility and mortality of *S. cervi* maintained under *in vitro* condition with varying concentrations of methanolic extracts of plants. Diethylcarbamazine (DEC) was used as a reference drug to compare the efficacy of plant extracts. The investigation revealed that the leaf extracts of *Artemisia ambrosifolia*, *Trifolium repens* and *Xanthoxylum rhetsa*, ripe berry extract of *Solanum myriscanthum*, and young fruit extract of *Carica papaya* possess consistent filaricidal activity.

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REFERENCES


