IN VITRO ANTHELMINTIC ACTIVITY OF ACTINOPTERIS RADIATA (SW.) LINK.

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Abstract
Aqueous and ethanolic whole plant extracts of Actinopteris radiata (Sw.) Link (Family-Actinopteridaceae) was investigated for anthelmintic activity against Haemonchus contortus, with varied drug concentration (25, 50, 100) mg/ml and the time taken for paralysis and death of the worm was determined. The in-vitro anthelmintic activity was compared with standard reference drug Piperazine citrate and control distilled water. The result showed ethanolic extract of higher concentration (100 mg/ml) had better efficacy than aqueous extract and lesser efficacy compared to Piperazine citrate.

Keywords: Anthelmintic activity, Actinopteris radiata, Pteridophytes and Haemonchus contortus.

INTRODUCTION
Parasitic disease mostly helminthiasis is the condition resulting from round worm infestations commonly Haemonchus contortus in small ruminants, is one of the major prevalent diseases in the world, particularly in the tropical countries, India (Soulsby, 2004; Urquhart et al, 1996). The use of anthelmintics, notably the development of resistant in helminthes (Wallier & Prichard, 1985) to various anthelmintic compounds and classes, as well as chemical residue and toxicity problems create awareness towards ethnic medicine. In general, recognition of the antigenic complexity of parasites has slowed vaccine development. Consequently there is an urgent and ever present need to control infections caused by H.contortus in ruminants. The frequent use of anthelmintics over many years has inevitably led to the development of drug resistance to each class in parasitic nematodes. H.contortus has been documented to be resistant to all three broad spectrum families of anthelmintics viz., benzimidazole, imidazothiazole and ivermectin. The emergence of resistance to anthelmintic drugs and the increased awareness of consumers about drug residues that potentially enter the food chain from ruminants, is one of the major problems associated with the use of anthelmintics (Waller & Prichard, 1985) to various anthelmintic compounds and classes, as well as chemical residue and toxicity problems create awareness towards ethnic medicine. In general, recognition of the antigenic complexity of parasites has slowed vaccine development. Consequently there is an urgent and ever present need to control infections caused by H.contortus in ruminants. The frequent use of anthelmintics over many years has inevitably led to the development of drug resistance to each class in parasitic nematodes. H.contortus has been documented to be resistant to all three broad spectrum families of anthelmintics viz., benzimidazole, imidazothiazole and ivermectin. The emergence of resistance to anthelmintic drugs and the increased awareness of consumers about drug residues that potentially enter the food chain from ruminants, is one of the major problems associated with the use of anthelmintics.

The whole part of fern Actinopteris radiata (Sw.) Link were collected from Thuckalay, Kanyakumari district (Southern Western Ghats) of Tamil Nadu and the herbarium was confirmed with the herbarium of Scott Christian College, Nagercoil and a sample specimen was preserved in A.V.V.M Sri Pushpam College, Thanjavur.

Preparation of plant extracts
Fresh leaves, stems and roots were washed in running tap water and cut into small pieces. Firstly, the plant materials were shade dried and then in Hot air oven at 55-60°C. Dust was prepared by pulverizing the dried leaves, stems and roots with the help of mixer. A 25- mm, mesh diameter sieve was used to obtain fine dust and preserved them into airtight plastic container, labelled, till their use in extract preparation. 10 gram of dust were taken in a 500 ml beaker and separately mixed with 100 ml ethanol. Then the mixture was stirred for 30 minutes by a magnetic stirrer (1000 rpm) and left stand for next 24 hrs. The mixture was then filtered through Whatman filter paper, No 1. The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent from filtrate in a water bath at 50°C for ethanol up to final volume of 10 ml (Sujon et al, 2008). After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labelled bottle and stored in refrigerator until their screening for anthelmintic property. Similar
procedure was adopted for the preparation of aqueous extract as per (Semwal & Farswan, 2012).

Worm collection and authentication
Adult live nematodes, *Haemonchus contortus* were collected from Perambur slaughter house, Chennai, from the g/l tracts (abomasm) of Sheeps. They were opened in a plastic bucket separately and the contents were washed up in tap water. The process was repeated for several times until the sediment becoming transparent. Then the adult g/l worms were collected with the help of a needle and placed in a petridish containing PBS (Phosphate Buffer Saline). Petridish containing the worms was kept in incubator at 38°C until required for experiment on the same day. In-vitro screening with pharmacological preparations (Ethanolic and aqueous extracts) of *Actinopteris radiata* was performed using *Haemonchus contortus* (Sujon et al, 2008). The ethanolic plant extracts were used at various concentrations i.e., 25 mg/ml (2.5%), 50 mg/ml (5%) and 100 mg/ml (10%), distilled water (control) and reference standard Piperazine citrate (2.5%, 5% and 10%) using adult nematode worms (n=6) in petridish. Observations were made for time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour. Statistical analyses were carried out and the results were expressed as mean ± SEM (Sourov et al, 2014; Sreejith et al, 2013).

RESULTS AND DISCUSSION

From the observation made aqueous and ethanolic extracts exhibited more potent activity at higher concentration (100 mg/ml) against *Haemonchus contortus*. Evaluation of anthelmintic activity was compared with standard Piperazine citrate. The ethanolic extract of *Actinopteris radiata* produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death (Table 1 and Figure 1). The aqueous extract showed significant activity. However in the present study, it was observed that ethanolic extract of *Actinopteris radiata* have exhibited positive and potent response than the aqueous extract and least response compared to the standard Piperazine. The order of activity was ethanol extract greater than aqueous extracts, confirmed to the study of (Semwal & Farswan, 2012) in Diplazium esculentum. The activity revealed concentration dependence nature of the different extracts.

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>Conc (mg/ml)</th>
<th>Time taken for paralysis (minutes)</th>
<th>Time taken for death (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Standard)</td>
<td>25</td>
<td>24.333 ± 1.382</td>
<td>57.500 ± 1.962</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>18.000 ± 1.095</td>
<td>37.500 ± 3.074</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.833 ± 0.792</td>
<td>23.833 ± 1.621</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>25</td>
<td>45.500 ± 1.384</td>
<td>99.833 ± 2.496</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>38.167 ± 1.249</td>
<td>75.167 ± 1.869</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>31.667 ± 1.249</td>
<td>61.833 ± 1.765</td>
</tr>
</tbody>
</table>

Table (1 ) Anthelmintic activity of whole plant extracts of *Actinopteris radiata*

CONCLUSION

It could be conclude that the ethanolic extract showed more potent anthelmintic activity. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of *Actinopteris radiata* as an anthelmintic drug.

References
