Evaluation of in Vitro Anthelmintic Activity of Bryophyte Pottia Lanceolata

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Abstract
The aqueous extract of Bryophyte Pottia lanceolata was investigated for anthelmintic activity using Pheretima posthuma (earthworms), Raillietina tetragona (tapeworms) and Ascaridia galli (roundworms). Various concentrations (10, 20 and 50 mg/ml) of plant extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug and distilled water as control. Significant anthelmintic activity at the concentration of 20 mg/ml was observed. The result shows that aqueous extract possesses vermicidal activity and found to be effective natural anthelmintic.

Keywords
Bryophyte, vermicidal, anthelmintic activity

Introduction
Infections with helminth are among the most widespread infections in humans and other domestic animals affecting a large number of world population. The majority of these infections due to worms are generally restricted mainly to the tropical regions and occurrence is accelerated due to unhygienic lifestyle and poverty also resulting in the development of symptoms like anaemia, eosinophilia and pneumonia. Helminthosis is a disease of animals caused by gastro-intestinal nematodes and has been recognized as constituting a major constraint to a profitable production of livestock throughout the world particularly to poor livestock owners of developing countries (Cheijina, 2001; J.K. Chamuah et al, 2013). The knowledge of plants, herbs and spices and their role in promoting health is increasing. If the safety and efficacy of these medicinal plants could be ascertained, they could be an alternative and effectively cheaper approach to control helminthosis in animals (Yadav et al, 2006; Athanasiadou S, 2007).

In their prevalence studies, (Permin et al, 1999) revealed a higher risk of helminth parasite infections in free range systems of poultry. Helminth parasites of poultry are usually controlled by repeated anthelmintic treatments of the flock. Regular and preventive use of such chemically synthesized drugs is not compatible with organic regulations and therefore effective preventive management strategies need to be developed in poultry, than in ruminants due to epidemiological differences of the helminth species involved (Kaushik et al 1974; Maurer et al, 2007).
Bryophytes are simplest non-vascular land plants with undifferentiated parts, they are more advanced than aquatic algae. They usually grow on tree trunks, leaves, figs and fallen logs in the form of dense green mats. These green mats exhibit light greenish to fluorescent green color and has velvety appearance. In human dwellings these tiny bryophytes grow abundantly on old damp walls. In current study an attempt has been made to evaluate antihelminthic potential of bryophytes belonging to genus *Pottia*, commonly found in the region of Telanagana.

Alternate treatments for gastrointestinal helminthes in poultry have been investigated by several authors, like (Lal *et al.*, 1976; Javed *et al.*, 1994; Tagbota S, Townson, 2001; Singh and Nagaich, 2002; Shivkumar et al 2003; Mali *et al* 2008) However, significantly validated data on the efficacy of herbal treatments against *Ascaridia galli* remain scarce and often limited. *Ascaridia galli* infection causes various forms of damage in the host intestinal and liver tissues, like haemorrhages and connective tissue hyperplasia. Treatment with Piperazine adipate causes histopathological changes and adverse tissue destruction,( Javed I, Akhtar MS, Rahman ZU, Khaliq T, Ahmad M, 1994 ). Extracts of *A sativum* and *Piper longum* have shown good hepatoprotective activity in rats (Rege N,Dhanukar S,Karandikar SM., 2011). When we work with nature we have on our side a creative and not a destructive intelligence. The life force in the organism will eagerly take up the natural substances and utilize them in no uncertain manner.

Apart from these natural extracts, certain other extracts such as algae, bryophytes and pteridophytes are also known to treat various pathogens. Phytochemical and anthelminthic evaluation of *Lantana camara (1)var Aculeate* leaves against *Pheritima posthuma* by jithendra patel *et al* volume 2, issue 1,pp 11-22, 2011 in Journal of global trends in pharmaceutical sciences. Anti-microbial activity and preliminary phytochemical screening of epiphytic moss *Stereophyllum ligulatum* by P kumar et al, International Journal of Pharma and Biosciences, volume 2/issue 4/2011, suggests that bryophytes can be effective against certain pathogens and so we speculate that bryophytes can also be effective anthelminthic agents against certain helminthes of poultry and also of sheep. The present study is an attempt to test the efficacy of bryophyte extract as an effective anthelminthic.

Materials and methods

Plant Materials

The Bryophyte, *Pottia lanceolata* was collected from Osmania University College for Women, Koti, Hyderabad and Golkonda Fort, Hyderabad, Telangana ,India. The plant material was taxonomically identified by the taxonomists of Botanical Survey of India, Hyderabad. A voucher specimen has been preserved in our laboratory for future reference. The plant material was dried in shade, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.
Preparation of extract

Aqueous extract (Maceration method)

The bryophyte sample was cleaned removing soil debris by thorough washings with water, dried in shade, powdered and preserved. The collected bryophyte was extracted using water by cold maceration technique and dissolved in water prior to use.

Experimental Animals

Adult *Pheretima posthuma* (earthworm), *Ascaridia galli* (roundworm) and *Raillietina tetragona* (tapeworm) were used to evaluate anthelmintic activity in vitro. Earthworms were collected from moist soil and washed with normal saline and used for the anthelmintic study. The earthworms of 5 cm in length and 0.2 cm in width were used for all the experimental protocol. Roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter. These intestines were then dissected. The average size of tapeworm was 6cms and roundworms were 5cms.

Earthworms and helminths were identified in Dept. of Zoology, UCW, Koti, Hyderabad.

Drugs

Piperazine citrate (Glaxo Smithkline) was used as standard anthelmintic during the experimental protocol.

Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al 2001. The assay was performed in vitro using adult earthworm *Pheretima posthuma* as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity and on poultry parasites *Ascaridia galli* and *Raillietina* species for screening of anthelmintic drug. Test samples of the extract were prepared at the concentrations, 10, 20 and 50 mg/ml in distilled water and six worms i.e. *Pheretima posthuma, Ascaridia galli* and *Raillietina tetragona* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg/ml) was used as reference standard and double distilled water as control. This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water.

All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.
TABLE – 1
Anthelmintic activity of Bryophyte *Pottia lanceolata* aqueous extract

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Groups</th>
<th>Concentration</th>
<th><em>Pheretima posthuma</em></th>
<th><em>Ascaridia galli</em></th>
<th><em>Raillietina tetragona</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mg/ml</td>
<td>Time taken for paralysis in minutes (mean and SEM)</td>
<td>Time taken for death in minutes (mean and SEM)</td>
<td>Time taken for paralysis in minutes (mean and SEM)</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>Distilled water</td>
<td>30±0.44</td>
<td>65±1.10</td>
<td>22±1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20±.46</td>
<td>48±1.12</td>
<td>14±1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10±0.55</td>
<td>60±0.83</td>
<td>08±1.36</td>
</tr>
<tr>
<td>2</td>
<td>Bryophyte extract</td>
<td>10</td>
<td>30±0.44</td>
<td>65±1.10</td>
<td>22±1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20±.46</td>
<td>48±1.12</td>
<td>14±1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>10±0.55</td>
<td>60±0.83</td>
<td>08±1.36</td>
</tr>
<tr>
<td>3</td>
<td>Standard Piparazine citrate</td>
<td>10</td>
<td>22±1.30</td>
<td>50±0.11</td>
<td>15±1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10±0.55</td>
<td>60±0.83</td>
<td>08±1.36</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSIONS:

From the observations, higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for all types of worms. Aqueous extract of Bryophyte exhibited anthelmintic activity in dose-dependent manner showing maximum efficacy at 20 mg per/ ml concentration for all three types of worms. The plant extract exhibited more potent activity at lowest concentration (10 mg/ml) against *Ascaridia galli*. Anthelmintic activity of the extract was compared with the standard drug Piparazine citrate (Table.1). From the above results, it can be concluded that Bryophyte extract exhibited significant anthelmintic activity. Therefore, further study must be carried out to get actual benefit from this important medicinal plant.
Reference


