INVESTIGATION OF IN-VITRO ANTHELMINTIC ACTIVITY OF JUSTICIA TRANQUIBAREINSIS

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ABSTRACT

Ethanolic extract of Justicia tranquibariensis was taken for anthelmintic activity against Indian earthworm (Pheretima posthuma), Roundworm (Ascaridia galli) and Tapeworms (Raillietina spiralis). Four different concentrations (20, 40, 80 and 100 mg/ml) of ethanolic extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/ml) was used as reference standard and double distilled water as a control group. The anthelmintic activity of Justicia tranquibariensis root extract has therefore been demonstrated for the first time.

KEYWORD: Anthelmintic, Justicia tranquibariensis, Pheretima posthuma, Ascaridia galli

INTRODUCTION

Justicia tranquubariensis (Acanthaceae) is a small shrub, which is widely distributed in the southern parts of India. Root is perennial, somewhat granulated, stem in young plants erect, in old ones uncertain, branches annual, small, stagling, round, a little downy, joined and swelled above the joints, from 1-2 ft long, leaves opposite, short, oval, round, entire, pretty smooth. Justicia tranquubariensis plant parts yielded several lignans, phytosterols, brassicasterol, campesterol, ergostadienol, stigmasterol, spinasterol, isofucostil and a sterol glycoside, betasitosterol –O–glycosides. Justicia have been used in the traditional system of medicines for the treatment of fever, pain, inflammation, diabetes, diarrhoea and liver diseases. They also possess anti-inflammatory, anti-allergic, anti-tumoral, anti-viral, anthelmintic and analgesic activities. The leaf juice of J.tranquebariensis has been used to treat jaundice and leaf paste is applied over affected area to treat skin diseases. Helminth infections are the most widespread infections in humans, distressing a huge population of the
world. Parasitic diseases cause ruthless morbidity, affecting principally population in developing countries. The gastro-intestinal helminthes are becoming resistant to currently available anthelmintic drugs; therefore there it is a fore most problem in treatment of helminthes disease. Hence an increase in demand towards natural anthelmintics is there. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro. The objective of the present research was to prove traditional anthelmintic use of the plant.

Plant material
The roots of *Justicia tranquibariensis* was procured from Chennai in January and February months of 2015. The plant material was authenticated by National institute of Herbal Science (PARC/2015/463), West Tambaram, Chennai. The plant material was size reduced, dried in sunlight, pulverized, passed through sieve no. 40, stored in air tight container and used for further extraction.

Preparation of extract
Powdered material of *Justicia tranquibariensis* roots (200 gm) was kept for maceration with 1000 ml of distilled water for 48 hrs. The extract was double filtered by using muslin cloth and concentrated by evaporation using rotary evaporator. The extract was dried and used.

Experimental animals
Adult earthworms (Pheretima posthuma), Roundworm (Ascaridia galli) and Tapeworms (Raillietina spiralis) were used to evaluate anthelmintic activity in vitro. Earthworms were collected near the swampy water along Edanad temple road, Chengannur. 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. The intestines were then dissected and worms were collected and kept in normal saline solution. The average size of earthworm was 5-9 cm, average size of round worm was 4-8 cm and average size of tapeworm was 5-8 cm. Services of veterinary practitioners were utilized to confirm the identity of worms.

Drugs and chemicals
Piperazine citrate (Glaxo Smithkline) was used during the experimental protocol.
Anthelmintic activity
The anthelmintic assay was carried out as per the method of Ajaiyeoba et al\(^1\). The assay was performed in vitro using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity. Use of *Ascaridia galli* and *Raillietina* species as a suitable model for screening of anthelmintic drug was advocated earlier. Test samples of the extract were prepared at the concentrations, 20, 40 and 80 mg/ml in distilled water and six worms i.e. *Pheretima posthuma*, *Ascaridia galli* and *Raillietina spiralis* 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 (10mg/ml) was used as reference standard and 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 except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (500\(\degree\)C). All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

RESULTS AND DISCUSSION
From the observations made, higher concentrations of the extract exhibited paralytic effect much earlier and the time to death was shorter for all the three types of worms. Ethanolic extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ ml concentration, for all three types of worms. Extract exhibited more potent activity at lower concentration (10mg/ml) against earth worms. Evaluation of anthelmintic activity was compared with reference standard Piperazine citrate (Table.1). Therefore further studies must be carried out so that the public gets actual benefit from this important medicinal plant.
Table 1: In – vitro anthelmintic activity of Ethanolic root extract of *Justicia tranquibariensis*

<table>
<thead>
<tr>
<th>S/No:</th>
<th>Groups</th>
<th>Concentration mg/ml</th>
<th><em>Pheretima posthuma</em> groups</th>
<th><em>Ascaridia galli</em> groups</th>
<th><em>Raillietina spiralis</em> groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Time taken for Paralysis (P) in mins Mean &amp; SEM</td>
<td>Time taken for Death (D) in mins Mean &amp; SEM</td>
<td>Time taken for Paralysis (P) in mins Mean &amp; SEM</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>2</td>
<td>Aqueous extract</td>
<td>20</td>
<td>40±0.5</td>
<td>65±0.34</td>
<td>46±0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>32±0.3</td>
<td>43±0.41</td>
<td>39±0.39</td>
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<tr>
<td></td>
<td></td>
<td>80</td>
<td>20±0.33</td>
<td>28±0.22</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>11±0.21</td>
<td>19±0.17</td>
<td>15±0.19</td>
</tr>
<tr>
<td>3</td>
<td>Piperazine citrate</td>
<td>10</td>
<td>22±0.46</td>
<td>35±0.48</td>
<td>18±0.51</td>
</tr>
</tbody>
</table>

**REFERENCES**


