ANTIHELMINTIC ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF EUPHORBIA THYMIFOLIA LINN

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ABSTRACT: Helminthes are recognized as a major problem to livestock production throughout the tropics. 1 Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results weaknesses, loss of appetite, decreased feed efficiency, reduced weight gain and decreased productivity. 2 Although some synthetic drugs are available to control such kind of infections but due to their high cost and untoward effects, the development of more effective and safe drugs from reasonably less expensive natural sources is our main consideration. This can rationally be approached through the study of indigenous traditional plant remedies. We here in explore scientifically the anthelmintic potential of three traditionally used medicinal plants of India and substantiate the folklore claims. In present communication methanolic and aqueous extracts of plant of Euphorbia thymifolia Linn. were investigated for their anthelmintic activity against Pheretima posthuma and Ascardia galli. Various concentrations were used in the bioassay, which involved paralysis and death time of the worms. Both the extracts showed significant anthelmintic activity.

KEY WORDS: Anthelmintic, Ascardia galli, Pheretima posthuma, Euphorbia thymifolia Linn, Piperazine citrate.

INTRODUCTION

Euphorbia thymifolia Linn is commonly known as dudhhi & is grown in India. It belongs to family Euphorbiaceae. The plant is bitter, acid, sweet, thermogenic, and laxative, diuretic. It is useful in vitiated condition of constipation, helminthiasis, and ringworm, skin diseases and leprosy. 3 The leaves and seeds are given in worm cases and in certain bowel affections of children & they are considered stimulant and laxative. 4,5 Antiviral activity is proven in experiment & Antimicrobial activity is reported. 6 Euphorbia thymifolia Linn. is one of the important multipurpose species of desert and arid regions of the Indian subcontinent. It provides vegetative cover in dry, hot, sandy desert areas where little else grows and is an extremely hearty species. Altitude range: 300-1200 mm, Mean annual rainfall: 100-750 mm, Mean annual temperature: 25-31° C, Soil, it prefers alkaline, sandy and gravelly soils, thriving on shallow, hard soils and rocky outcrops. 6 Phytochemical screening of the extracts revealed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins and steroids. Purpose of the study is to evaluate anthelmintic potential of plant Euphorbia thymifolia linn.

MATERIALS AND METHODS

Plant material:
The plant Euphorbia thymifolia linn. were collected from Sangli district of Maharashtra in June 2008 and authenticated by Dr U. S. Yadav, Dept. of Botany, Willingdon College Sanglis, Maharashtra.

Preparation of extracts:
The plant of were shade dried, powdered and subjected to successive solvent extraction petroleum ether (60-80%) chloroform, methanol in soxhlet extractor and cold maceration of the successive marc in water. Extracts were vacuum dried. The preliminary phytochemical investigation was carried out of methanol and aqueous extracts.

Animals
Indian adult earthworms (Pheretima posthuma) were collected from water logged areas and Ascardia
galli (nematode) worm were obtained from freshly slaughtered fowls (Gallus gallus). Both worm types were identified at the Department of Zoology, Willingdon College, Sangli.

**Evaluation of anthelmintic activity:**

The anthelmintic assay was carried as per method of Ajaiyeoba et al with minor modifications. The anthelmintic activity was evaluated on adult Indian earthworm *Pheretima posthuma* worm due to its anatomical and physiological resemblance with the intestinal round worms parasites of human beings *Ascardia galli* (nematode) worms are easily available in slaughtered fowls and it can be used as a screening model for anthelmintic drugs as advocated earlier. Three different concentrations, each of crude alcoholic and aqueous extract (10, 50, 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. This was done for both type of worms. Observation were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis (P) in min was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; time of death (D) in min was recorded after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Piperazine citrate (10 mg/ml) was included as reference compound.

**RESULTS AND DISCUSSION**

Preliminary phytochemical screening of plant of *Euphorbia thymifolia* Lind showed the presence of diterpenoids, flavonoids, steroids, tannins and resins. As shown in table-I, methanolic and aqueous extract exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration. The alcoholic extract of *Euphorbia thymifolia* Lind caused paralysis of 9.66 min. and time of death of 30.5 min. while aqueous revealed paralysis of 8.66 and 33.33 min. respectively against the earthworm *Pheretima posthuma*. The reference drug Piperazine citrate showed the same at 18.83 and 60.33 minutes, respectively. *Ascardia galli* worms also showed sensitivity to the methanolic and aqueous extract of *Euphorbia thymifolia* Lind. The methanolic extract caused paralysis in 10.42 min, death in 31.75 min and the aqueous extract displayed P and D in 9.10 and 30.22 min, respectively, at higher concentration of 100 mg/ml. Piperazine citrate did the same at 15.17 and 41.67 min.

<table>
<thead>
<tr>
<th>Test subs</th>
<th>Concentrations</th>
<th>Time taken for paralysis (P) and death (D) (mg/ml) of worms in mins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>D</td>
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<tr>
<td>Control</td>
<td>-</td>
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</tr>
<tr>
<td>Alcohol extract</td>
<td>10</td>
<td>26.5 ± 0.56</td>
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<tr>
<td></td>
<td>50</td>
<td>17.0 ± 0.56</td>
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<tr>
<td></td>
<td>100</td>
<td>9.66 ± 0.49</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>10</td>
<td>29.17 ± 0.47</td>
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<tr>
<td></td>
<td>50</td>
<td>19.67 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>08.66 ± 0.33</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>10</td>
<td>18.83 ± 0.60</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM from six observations.
DISCUSSION

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. The leaf extract of *Euphorbia thymifolia linn.* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml in shorter time as compared to reference drug Piperazine citrate. Phytochemical screening of the extracts revealed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins and steroids. Tannins were shown to produce anthelmintic activities chemically tannins are polyphenolic compounds. It is possible that tannins contained in the extracts of *Euphorbia thymifolia linn.* produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. Further studies are in process to identify the possible phytocconstituents responsible for anthelmintic activity.

ACKNOWLEDGEMENT

We all authors are thankful to our beloved Principal Prof. J.S. Shete for his kind support and guidance in our work.

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