Anthelmintic activity of methanolic extract of *Euphorbia milii*

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Abstract
The purpose of this study was to examine the anthelmintic activity of *Euphorbia milii* (*E. milii*) leaves, which were selected as a different source of potent treatments for nematodiasis. The anthelmintic activity of *E. milii* was tested in adult *Pheretima posthuma* Indian earthworms. The reference standard was albendazole (10 mg/ml), while the control treatment was normal saline (10 ml). The extracts were tested at various methanolic concentrations (20 mg/mL, 40 mg/mL, 80 mg/mL, and 100 mg/mL).

To report the earthworms' paralysis and death time, the data were stated in terms of time in minutes. The study's observations have led to anthelmintic action, supporting traditional use of both plants when compared to the standard.

Keywords: *Euphorbia milii*, paralysis, anthelmintic, nematodiasis

Introduction
Around two billion people worldwide have intestinal parasitic nematodes. Currently, chemotherapy is the major method used to manage infections due to the lack of vaccinations for human intestinal nematodes, although resistance is becoming a bigger issue. Therefore, there is a great need for the discovery and development of new anthelmintic medications, particularly those with novel mechanisms of action. An effective source of therapies, including anthelmintic therapy, are medicinal plants [1]. They have a long history of usage and are generally safe (if not, their toxicity is well-known). However, it has not yet been determined which chemicals in the majority of medicinal plants are effective against nematodes [2].

The deciduous shrub *Euphorbia milii* Des Moul is commonly grown for ornamental purposes. Belonging to family Euphorbiaceae. In English, it is usually referred to as a "Christ-plant [3]". Since ancient times, it has been frequently utilised as a traditional remedy. The plant has historically been used as a parasite and wart therapy as well as an anti-inflammatory, antioxidant, antispasmodic, anticancer, and antitussive agent [4]. Flavonoids, triterpenes, β-sitosterol, lupeol, cycloartenol, β-amyrin acetate, and euphol are the main chemical components found in *Euphorbia milii* [5].

The purpose of this study is to assess the anthelmintic activity of *Euphorbia milii* against a *Pheretima posthuma* model.

Plant description
A thorny, multi-branched evergreen shrub with a height range of 60 to 90 cm, *Euphorbia milii*. It grows in full sun and dry to moderately wet, well-drained soil. In the winter, it is susceptible to temperatures lower than 35°F. Even though *Euphorbia milii* can withstand dryness and poor soils, especially rocky-sandy soils, it may benefit from routine applications of mild moisture since it will bloom better and lose fewer leaves. Particularly in the winter, wet soils can be deadly. It works best in areas with sufficient airflow [6].

Material and Methods
Collection of Plant and Authentication
*Euphorbia milii* was collected at the JNTUH Campus in Kukatpally, Hyderabad, Telangana, in September 2022. Dr. A. Vijaya Bhasker Reddy, Assistant Professor, Department of Botany, University College of Sciences, Osmania University, Hyderabad, Telangana State-500007, authenticated and taxonomically recognized the plant specimen and preserved it for future use under voucher number OUAS-85.
Preliminary phytochemical screening
A qualitative phytochemical examination revealed, the presence of several secondary metabolites, such as proteins, amino acids, carbohydrates, alkaloids, phenol, flavonoids, saponins, and glycosides.

Preparation of Plant Extract
The leaves were shade-dried (2 kg) and then crushed into a coarse powder (30 gr) Euphorbia milii leaf material was then placed in an airtight container. The powder was defatted, then by using a Soxhlet apparatus, the powdered material was put in a thimble (25 gm), and methanolic extraction was carried out at 70 °C for 12 hours. The extract was put to a rotary evaporator at decreased pressure in order to separate the solvent from the solute and produce dried plant extract, which was then stored in a desiccator for later use. The crude extract from the dried methanolic extract was carefully quantified at 20mg/mL, 40mg/mL, 80mg/mL, and 100mg/mL before being dissolved in distilled water to the required concentrations for the experiment.

Worm Collection
The Indian earthworm Pheretima posthuma (Annelida) was obtained from waterlogged areas of soil, with an average size of 6-8 cm. They were rinsed with tap water to remove the adhering dirt. Due to its morphological and physiological similarities to the intestinal roundworm parasites that infect humans, the adult Indian earthworm Pheretima posthuma was used for the experiment. The readily available Pheretima posthuma worm serves as an ideal model for testing anthelmintic medication.

Drugs Used: Albendazole as standard, normal saline as control, distilled water.

Anthelmintic Activity
Indian adult earthworms (Pheretima posthuma) of 3-5 cm length and 0.1-0.2 cm width were used to test the anthelmintic activity of Euphorbia milii leaves extracts. The earthworms were placed into six groups, each with earthworms equally divided into petri plate. All Euphorbia milii extracts were diluted in distilled water to a volume of 10 mL. Before starting the experiment, all of the extracts and the reference drug solution were freshly prepared. Various extracts and the standard medication solution were put into various Petri dishes.
All earthworms were rinsed in regular water before being placed in 10 mL of the following formulation: Albendazole (10mg/mL). The methanol extract (concentrations of 20 mg/mL, 40 mg/mL, 80 mg/mL, and 100 mg/mL mg/mL). There were observations recorded as, to how long it took for worms to become paralyzed and die. The time for paralysis was recorded when no movement could be detected except when the worms were severely agitated. The worms died when they lost their motility.

Table 1: The anthelmintic activity of the methanolic extract of Euphorbia milii leaves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations</th>
<th>Paralysis time (min)</th>
<th>Death time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>10 mg/ml</td>
<td>9.20</td>
<td>13.50</td>
</tr>
<tr>
<td>Saline</td>
<td>10 ml</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Methanolic extract of Euphorbia milii</td>
<td>20 mg/ml</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>40 mg/ml</td>
<td>23.7</td>
<td>30.10</td>
</tr>
<tr>
<td></td>
<td>80 mg/ml</td>
<td>11.8</td>
<td>16.37</td>
</tr>
<tr>
<td></td>
<td>100mg/ml</td>
<td>7.32</td>
<td>11.00</td>
</tr>
</tbody>
</table>

Results
A preliminary phytochemical screening revealed the presence of triterpenes, steroids, tannins, alkaloids, phenol, flavonoids, saponins, and saponin and glycosides in the E. milii. These phytoconstituents may be the cause of some of their potent anthelmintic effects. According to the observations, the methanolic extract of E. milii leaf showed significant anthelmintic action when compared to the standard drug. According to the observations, 100 mg/ ml concentrations of total methanolic extracts of E. milii leaves exhibited significant anthelmintic action. Results are comparable to those of standard drugs. Albendazole According to Table 1, the anthelmintic activity was demonstrated by the methanolic extract of E. milii leaves.
At a dosage of 100 mg/ml, aqueous extracts of E. milii exhibit paralysis at 7.32 minutes and death at 11:30 minutes. Albendazole, the standard drug, causes paralysis at 9.20 min and death at 13.50 min.

Based on the results, it is concluded that all extracts of E. milii leaves show significant anthelmintic activity when compared to regularly used drugs and are equivalent to standard anthelmintic drugs. More research utilizing in vivo models is needed to carry out and demonstrate the efficacy and pharmacological justification for employing E. milii as an anthelmintic medication. The drug's phytochemical composition might be investigated further in order to determine the active ingredient responsible for anthelmintic action.

It is evident that the methanolic extract of E. milii leaves contains the active ingredients necessary for anthelmintic action.

Conclusion
In conclusion, the anthelmintic activity of methanolic extract (leaves) of Euphorbia milii has been confirmed, since the leaves shown action against the worms utilised in the study. In the future, it will be important to discover and isolate the active phytoconstituents responsible for the anthelmintic activity, as well as examine their pharmacological activities.

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