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**ABSTRACT**

Intestinal worms affect a host of individuals resulting in malnutrition, stunted growth, intellectual retardation and cognitive deficits. *Nerium indicum* Mill belonging to the family Apocynaceae is a wild plant known *kaner* in Hindi as well as exile tree, Indian oleander. Phytochemical study of the plant root extracts reveals to contain various phytoconstituents like glycosides, flavonoids, carbohydrates, tannins and steroids. Hence roots of the plant was collected, authenticated, powdered and extracted with various solvents by hot extraction to obtain petroleum ether, chloroform, & ethanol extracts and also cold maceration to obtain ethanol extract and evaluated for its anthelmintic activity on adult Indian earthworms (*Pheritina posthuma*), which have anatomical and physiological resemblance with the intestinal roundworms parasites of human beings. Four concentrations (10, 30, 50 and 100mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole was used as reference standard. Dose dependent activity was observed in all plant extracts; although at all concentration level Ethanolic extract exhibited more activity in terms of time taken for paralysis as well as death of worms in a less time as compared to other extracts. All extracts shows standard comparable activity at 50mg/ml and 100mg/ml concentration. Thus extracts exhibited anthelmintic activity and could be an inexpensive and readily available source of anthelmintic treatment.

**Keywords:**
Nerium indicum, Pheritina posthuma, Anthelmintic activity, Apocynaceae

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Introduction

Anthelmintic or antihelminthics are the drugs or the agents that destroy or cause the expulsion of such parasitic intestinal worms and helps to treat Helminthiasis, one of the most common infections in humans and cattle. Helminthiasis is a macroparasitic disease of humans and animals in which a part of the body is infected with parasitic worms such as pinworm, roundworm, or tapeworm. This infection is highly prevalent particularly in third world countries due to poor management practices. Helminthiasis can have immunodilatory effects on the host, with implications for any co infecting pathogens. A number of medicinal plants have been used to treat parasitic infections in man and animals. Albendazole is the first drug of choice for the treatment of worm infections.

_Nerium indicum Mill_, (Syn. _N. odorum_ soland) is commonly known as ‘kaner’ belonging to family Apocynaceae. It is widely distributed in Mediterranean region and subtropical Asia but is native of Indian subcontinent and cultivated elsewhere. All parts of the plant are reputed as therapeutic agent and have been used in folklore in a variety of ailments. The leaves and bark are used as heart tonic, diuretic, expectorant, diaphoretic and emetic. Roots boiled in water are considered helpful when applied externally in skin complaints, herpes and ringworm infection. Leaf juice in very small doses is given in snake and other venomous bites. Juice of young leaves is effective in opthalmia with lacrimation. Infusion of the leaves is abortive. Root paste is used in haemorrhages various types of cancer, Ulceration and leprosy. With this background, present study was undertaken to explore in-vitro anticancer and antioxidant activity of _Nerium indicum_, Mill.

Material and Method

Plant material

The roots of _Nerium indicum_, Mill (Apocynaceae) collected from the Rural Area of Chakur, Tq. Chakur, Dist. Latur, Maharashtra, India, was authenticated by Dr. B.D. Gachande P.G. Department of Botany, N.E.S. Science College, Nanded, Maharashtra, India (specimen-S-4/NPC/2011-12) as _Nerium indicum_, Mill, and identified by morphological and microscopical characteristics as _Nerium indicum_, Mill (Apocyanaceae). (Fig 1 & 2). The selected experimented animals (earthworms) is authenticated by Dr. R. P. Mali, Head and Research Guide, Department of Zoology, Yeshwant Mahavidyalaya, Nanded, as it _Pheritima posthuma_ (Indian Earthworm). (Fig 3)

Qualitative chemical investigation

The plant material was extracted successively by petroleum ether (60-80°C), Chloroform and Ethanol by hot percolation using soxhlet apparatus and Ethanol by cold maceration method. All extracts were subjected to proximate chemical analysis.
Fig 1: Roots: Used Plant Material of *Nerium indicum*, Mill

Fig 2: Specimen of the *Nerium indicum*, Mill plant
**Fig 3: Pheritima posthuma** (Indian Earthworm)

Proximate chemical analysis

Extracts were screened by applying general chemical tests for alkaloids, glycosides, Sugars, Phenolic compound, Sterols, Tannins, proteins, Saponin and free amino acids the positive result quote in Table 1

**Table 1: Qualitative Determination of Extracts**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Tests</th>
<th>PE-NI</th>
<th>CH-NI</th>
<th>Et-NI</th>
<th>Etm-NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) present, (-) Absent  
PE-NI= Petroleum ether (60-80 °C) Nerium indicum extract, CH-NI= Chloroform Nerium indicum extract, Et-NI= Ethanolic Nerium indicum extract Etm-NI= Ethanol Nerium indicum Maceration extract.
Pharmacological screening

Anthelmintic activity

Test animals

*Pheritima posthuma* (Indian Earthworm)

Test sample and standard

Petroleum ether (60-80°C), Chloroform and Ethanolic extract by hot percolation using soxhlet apparatus and Ethanolic extract by cold maceration method (10 mg/ml, 30 mg/ml, 50 mg/ml & 100 mg/ml) and Albendazole suspension (10 mg/ml, 30 mg/ml, 50 mg/ml & 100 mg/ml).

Table 2: Anthelmintic activity of *Nerium indicum*, Mill root extracts.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Extracts</th>
<th>Concentration (mg/ml)</th>
<th>Paralysis Time (min)</th>
<th>Death Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>01</td>
<td>PE-NI</td>
<td>45.00±13</td>
<td>36.00±12</td>
<td>30.00±12</td>
</tr>
<tr>
<td>02</td>
<td>CH-NI</td>
<td>65.00±7</td>
<td>46.00±11</td>
<td>40.00±10</td>
</tr>
<tr>
<td>03</td>
<td>Et-NI</td>
<td>54.5±17</td>
<td>33.5±8.5</td>
<td>28.5±8.5</td>
</tr>
<tr>
<td>04</td>
<td>Etm-NI</td>
<td>64.00±12</td>
<td>51.00±90</td>
<td>40.00±12</td>
</tr>
<tr>
<td>05</td>
<td>Albendazole</td>
<td>37.5±2.5</td>
<td>34.5±0.5</td>
<td>37.5±2.5</td>
</tr>
<tr>
<td>06</td>
<td>Control</td>
<td>Normal saline</td>
<td>No Effect</td>
<td>No Effect</td>
</tr>
</tbody>
</table>

Each value presented as Mean±SEM (P<0.05) one way ANOVA followed by Dunnett’s test n=3, PE-NI= Petroleum ether (60-80 °C) Nerium indicum extract, CH-NI= Chloroform Nerium indicum extract, Et-NI= Ethanolic Nerium indicum extract Etm-NI= Ethanol Nerium indicum Maceration extract.
Experiment

Indian adult earthworms of 3-7 cm length and 0.1-0.2 cm width were used for anthelmintic activity because of their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. These are collected from moist soil and washed with normal saline solution to remove all fecal matter. In the experiment, earthworms were released the solution of albendazole, petroleum ether (60-80°C), Chloroform and Ethanol extracts (hot percolation using soxhlet apparatus) and Ethanol extract (cold maceration method) in a concentration of 10 mg/ml, 30 mg/ml, 50 mg/ml & 100 mg/ml. Observations were made for the time taken to paralyze and death of individual earthworms. Time for paralysis was noted when no movement could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colours. All readings were expressed as mean and standard error of mean (SEM) n=3.

Statistical analysis

All values shown as mean ± SEM n=3. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett’s test P<0.05 was considered statistically significant.

Result and Discussion

Preliminary Phytochemical screening of crude extracts of Nerium indicum, Mill (Roots) showed the presence of phytoconstituents such as Protein, Flavonoids, Carbohydrates, Tannins and Steroids. The result of the present

Fig 4: Anthelmintic activity of PE-NI extract10mg/ml, B- 30mg/ml, C-50mg/ml, D-100mg/ml, E- Standard, F-Control
Fig 5: Anthelmintic activity of CH-NI extract 10mg/ml, B- 30mg/ml, C-50mg/ml, D-100mg/ml, E-Standard, F-Control

Fig 6: Anthelmintic activity of Et-NI extract A-10mg/ml, B- 30mg/ml, C-50mg/ml, D-100mg/ml, E-Standard, F-Control

study reveals that the roots of *Nerium indicum*, Mill showed significant anthelmintic activity as compared to the standard drug albendazole at a concentration of 10 mg/ml, 30 mg/ml, 50 mg/ml & 100 mg/ml (Table 2 and Fig 4 to 7).
Fig 7: Anthelmintic activity of Etm-NI extract A-10mg/ml, B- 30mg/ml, C-50mg/ml, D-100mg/ml, E-Standard, F-Control

Conclusion
Different extracts of *Nerium indicum* Mill root were screened for anthelmintic activity on Indian Earthworms (*Pheritima posthuma*). Albendazole was used as a reference standard for comparison of the anthelmintic activity. Observations were made for the time taken for paralysis and/or death of worms. Successive extract of roots of *Nerium indicum*, Mill showed significant anthelmintic activity on selected worms. Ethanolic extract found to be more active as compared to remaining extracts. The Ethanolic extract demonstrated paralysis as well as death of worms in a less time as compared to other extracts and albendazole at higher concentration (100mg/ml); although all extracts shows standard comparable activity at 50mg/ml and 100mg/ml concentration. Result of the study indicates plant extracts has a promising anthelmintic activity.
Reference


