INVESTIGATION OF IN-VITRO ANTHELMINTIC ACTIVITY OF ANETHUM GRAVEOLENS AGAINST PHERETIMA POSTHUMA

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ABSTRACT
Development of anthelmintic drugs leads to evaluation of medicinal plants as a source of anthelmintics. In view of this, an attempt has been made to study the anthelmintic activity of leaves of plant of Anethum graveolens. In the current study, experiments were conducted to evaluate the possible anthelmintic activity of alcoholic extracts of the leaves of plant Anethum graveolens. Various concentrations (20, 40, 60, 80, 100mg/ml) of alcoholic extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Also Albendazole used as a reference standard and saline as a control group.

KEY WORDS: Anethum graveolens (Dill), Anthelmintic.

INTRODUCTION
Helminthes infection are the important and among the most common infections in human being, affecting a large proportion of the world’s population. The disease is highly prevalent particularly in third world countries due to poor management practices. In developing countries they pose a large threat and public health and contribute to prevalence of anemia, malnutrition, eosinophilia, and pneumonia.¹ Anthelmintic or anthelmintic are drugs that expel parasitic worms (helminthes) from the body. They may also be called vermifuges (stunning) or vermicides (kill).² Anthelmintic are drugs that may act locally to expel out worms from the GIT. Most of the existing anthelmintic produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea. Hence there is an increasing demand towards natural anthelmintic. The whole plant of dill (Anethum graveolens) (Apiaceae) is used as anthelmintic agent traditionally to eradicate or reduce the number of helminthic parasites in the intestinal tract³. The Anethum graveolens has been
cultivated Mediterranean region, and found in many places such as India, China, Europe and United States. *Anethum graveolens* is commonly known as Dill and contains chemical constituents such as tannins, terpenoids, cardiac glycosides, flavonoids, phenolic compounds. Fruits of dill contain 1-4% essential oil comprising of major compounds: carvone (30-60%), limonene (33%), alpha-phellandrene (20.61%), including pinene, diterpene, dihydrocarvone, cineole, myrcene, Para myrcene, dillapiole, isomyristicin, myristicin, myristin, apiol and dillapiol. It is used for some gastrointestinal ailments such as flatulence, indigestion, stomachache and colic. Its common use in Ayurveda medicine is in abdominal discomfort, colic and promoting digestion. The fruit has an antispasmodic effect on the smooth muscle of the gastrointestinal tract with regard to central nervous system (CNS). The aerial parts of the plant are often cooked with fish to add flavor to it and stimulate the brain. The dill also used in traditional medicine for the properties such as, carminative, stomachic, diuretic, dill water is believed to have a soothing effect and is given to babies to treat gripe, relieve hiccups and colic. Dill has been reported to possess cardio protective, anti-hyperlipidaemic and anti-hypercholesterolemia. Antioxidant activity of the aqueous extracts of dill is comparable with alpha-tocopherol, ascorbic acid and quercetin. The essential oil produced from the seed oil is found effective against candidias in immunosuppressed mice. Also it is having an antimicrobial activity. With this objective this study focuses on the validation of the traditional use of *Anethum graveolens* as anthelmintic agent.

**MATERIAL AND METHODS**

**Collection and Identification**

The leaves of *Anethum graveolens* were collected in Karad, Satara District, and State of Maharashtra. These specimens were identified by in the Herbarium of Department of Pharmacognosy, Shree Santkrupa College of Pharmacy, Ghogaon

**Preparation of Extracts**

For the preparation of aqueous extract, aerial part was collected, shade dried At room temperature, pulverized and extracted with water by maceration process for 24 hrs. The extract was concentrated in a rotary flash evaporator and dried in desiccator.

**Test drug**

The leaves of plant *Anethum graveolens* was dried in sunlight and powdered coarsely. The powdered *Anethum graveolens* was extracted with different solvents (chloroform, alcohol, acetone, GAA, and ether) for 7 days. Then this extract was filtered and powder from each
solvent was dried in shade. And then a different concentration (20, 40, 60, 80, 100mg) of ethanolic extract solution was prepared by diluting the stock solution, in propylene glycol, using normal saline.

**Reference drug**

Albendazole was prepared by dissolving them in normal saline at a concentration of 20 mg/ml.

**Normal control**

Normal saline was prepared and used to treat the normal control group.

**Animals**

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. The earthworms of 9-10 cm in length and 0.3-0.4 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.

**Anthelmintic activity**

For the anthelmintic activity of plant extract of *Anethum graveolens*, Indian adult earthworms (*Pheretima posthuma*) of 9-10 cm in length and 0.2-0.4 cm in width were used. The animals were divided into seven groups containing six earthworms in each group. Different dilutions of drug extract (20, 40, 60, 80 and 100 mg/ml) has been made and then the volume was adjusted to 100 ml with normal saline water. All the dilutions of drug extract and standard from the above results, it is concluded that the aqueous extract of entire plant of *Anethum graveolens* have potent anthelmintic activity when compared with the conventionally used drug. Further studies using in vivo models are required to carry out and drug solution were freshly prepared before starting the experiments. Different extract dilutions and standard drug solution were poured in different petri dishes. All the earthworms were washed in normal saline before they were released into 10 ml of respective formulation. Observation were made for the time taken to paralyze (Paralysis was said to occur when the worm did not revive even in normal saline) and death (Death was concluded when the worms lost their motility followed with their body colors fading away) 10. All the results were expressed as a mean 1 animal in each Petri dish.
Table-1 *In vitro* anthelmintic activity of ethanolic extracts of *Anethum graveolens*:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment of extracts</th>
<th>Concentration (mg/ml)</th>
<th>Time taken for paralysis (min)</th>
<th>Time taken for death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Albendazole (Standard)</td>
<td>20</td>
<td>45±1</td>
<td>60±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>137±6</td>
<td>150±10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>125±5</td>
<td>142±8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>115±4</td>
<td>130±7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>110±2</td>
<td>122±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>90±1</td>
<td>105±4</td>
</tr>
<tr>
<td>3.</td>
<td>Alcohol</td>
<td>20</td>
<td>45±1</td>
<td>60±2</td>
</tr>
</tbody>
</table>

Comparison made between standard versus treated groups.

20mg/ml

![Image of 20mg/ml](image-url)

40mg/ml

![Image of 40mg/ml](image-url)
RESULT AND DISCUSSION

The observed response of worms in case of paralysis there was significant variation among the result produced by extract at different concentrations like 20, 40, 60, 80, and 100mg/ml. The alcoholic extract showed more significant effect on paralyzing worms. (Table I). This result may lend support for the traditional use of the plant as an anthelmintic. Preliminary phytochemical screening shows presence of glycosides flavonoids and tannins. Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators. Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite, and cause death. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the anthelmintic activity observed.

CONCLUSION

Throughout screening of literature available on *Anethum graveolens* it’s very useful in treating many diseases. The wormicidal activity of alcoholic extracts of whole plant of *Anethum graveolens* suggests that it is effective against parasitic infections of humans. The data presented in table and observations made there of lead to the conclusion that the different degree of helminthiasis of the different extracts are due to the level of tannins present in compound.
ACKNOWLEDGEMENT
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REFERENCES
1. Ravindra GM and Anita AM, Navrangapura A. review on anthelmintic plantsnatural
   product radiance, 2008: vol.7 (5), 466-475.
   activity of moringaoleifera seed oil - validation of traditional use, journal of advanced
3. Bairagi GB. Kabra AO. Mandade RJ. Anthelmintic activity of citrus medica l. leaves in
   indian adult earthworm, international journal of pharm tech research coden ; 2011, vol.
   3(2), 664-667
4. Heamalatha S, Swarnalatha S, Divya M, Gandhi LR, Ganga devi A, Gomathi E,
   pharmacognostical, pharmacological, investigation on anethumgraveolens, research
   journal of pharmaceutical, biological and Chemical sciences, 2011; volume 2, 564.
5. Dahiya P, Purkayastha S, phytochemical analysis and antibacterial efficacy Of dill seed
   oil against multi-drug resistant clinical isolates; Asian Journal of Pharmaceutical and