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Anthelmintic activity of *Nerium olender* flower extract in Indian adult earthworm

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ABSTRACT

Nerium olender popularly known as 'Indian olender' in India belongs to family Apocyanaceae. The objective of the present work was to identify the phytochemical constituents and also to evaluate the in vitro anthelmintic potency of the aqueous extract of Nerium olender flower using Indian earthworms (Pheretima posthumad). The various concentrations (15, 25, 50 & 100 mg/ml) of the aqueous extract were tested in vitro for anthelmintic potency by determination of time of paralysis and time of death of worm. Albendazole (15mg/ml) used as standard. The result of present study indicates Nerium olender potentiate to paralyze earthworm and also caused its death after some time. Thus, the present study demonstrates that Nerium olender is a potent anthelmintic.

Keywords: Death, *Nerium olender*, Paralysis, *Pheretima posthumad*.

INTRODUCTION

Nowadays the term "worms" is used clinically, it has a more restricted meaning and indicates various helminths. Helminthiasis is a macroparasitic disease observed in humans and animals in which a part of the body is infested with parasitic worms such as Roundworms (Nematodes), Tapeworms (Cestodes) or Flukes (Trematodes) figure no 1. Typically, the worms reside in the GI tract but may also burrow into other organs like liver (*Fasciola hepatica)*, lung (*Paragonimus westermani)*, muscle *(*cysticercosis), skin (*Strongyloides)*, lymph *(Wuchereria bancrofti)*, eye *(O. volvulus)*, brain *(Paragonimus sp)* and other tissues. Anthelmintics are drugs that destroy or expel parasitic intestinal worms from the body, by either vermifuges (stunning) or vermicides (killing). Most of the existing anthelminthics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea[1]. Since ancient times herbal drugs are used for the treatment of parasitic diseases in human without any side effects[2]. To eradicate the

side effects of the present allopathic drugs now scientist are moving towards the herbal drugs what our ancient peoples used. In this way to create the scientific evidence for the natural herbs *Nerium olender* flower selected for the anthelmintic activity.

Figure no 1: Types of helminthes

Nerium oleander belonging to family Apocyanaceae is an evergreen, shrub or small tree with white and red flowers both possess similar properties. The leaves bark and flowers are majorly used for the treatment of various diseases and functional disorders. The leaves are used for cardiovascular diseases, as well as for skin diseases. The leaves and roots have a number of active constituents including glycosides, terpenoids, sterols and other compounds [3]. It is an important herbal drug used as Analgesic [4], Anticonvulsant [5], Anti-anxiety [6], Antioxidant [7], Antidiabetic [8], Anticancer [9], Antibacterial [10], Anti fungal [11] and Insecticidal. Traditionally, *Nerium olender* was claimed as anthelmintic but scientifically it is not reveled still. Thus the present study was design to evaluate the *in vitro* anthelmintic activity of Aqueous extract of *Nerium olender* flower.

MATERIALS AND METHODS

Plant material:

The *Nerium oleander* (Apocynaceae) flower was collected from the city Thanjavur, Tamil Nadu south India in the month of September 2011. The plant was identified and authenticated (BSI/SRC/5/23/2011-12/Tech-1054) by Dr. G.V.S.Murthy, Head of office, Botanical Survey of India, Coimbatore, Tamil Nadu.

Extract Preparation:
The collected *Nerium oleander* flowers were washed thoroughly in water and air dried for a week at 35-40^{o}C and to remove the moisture content the flower was kept in hot air oven at 25^{0} C for 30 minutes. The dried flowers were pulverized in electric grinder and stored in air tight container for aqueous extraction by decoction method [12, 13]. The extracts were concentrated under reduced pressure using rotary vacuum evaporator.

Experimental animals:
The *in vitro* anthelmintic activity$_1$ were carried out in Indian adult earthworms (*Pheretima posthumad*) collected from moist soil and washed with normal saline to remove all fecal matter [14]. Easy availability and anatomical , physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids* of human beings [15], earthworms have been used widely for the initial evaluation of anthelmintic activity [16, 17]. Earthworms were identified by Dept. of Microbiology, PRIST University, Thanjavur, Tamil Nadu, India.

Administration of Extract*:*
Aqueous extract of *Nerium olender* flower at different concentration (15, 25, 50 & 100mg/ml) were prepared by diluting the stock solution, using normal saline and its final volume was made up to 10 ml. Six groups of approximately six earthworms individually in each group with equal size were released into 10 ml of desired concentration of extracts and drug.

Administration of Albendazole:
Albendazole was prepared by dissolving them in normal saline at a concentration of 15mg/ml [18].

Experimental Design:
Aqueous extract of *Nerium olender* flower at different concentration (15, 25, 50 & 100 mg/ml) were prepared by using normal saline (0.9% Nacl) and its final volume was made to 10 ml. Albendazole (15mg/ml) was used as a standard. The final volume of standard drug solution and different concentration of extracts were poured in different Petri dishes. Indian adult earthworms (*Pheretima posthumad*) collected from moist soil and washed with normal saline were used for anthelmintic activity. The anthelmintic assay was carried out as per the method of (Ajaiyeoba 2001) with minor modification [19]. The animals were divided into six group containing six earthworms in each group with equal size. Group I and II received normal saline and standard drug Albendazole (15mg/kg), Group III, IV, V and VI received different concentration of aqueous extract of *Nerium olender* flower. Earth worms were placed in 10 ml of desired concentration of drug and extracts. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor dipped in warm water (50^{0}c), followed with their body colors fading away) for evaluation of anthelmintic activity of *Nerium olender* extract [20].

Preliminary phytochemical screening:
The extract was used for preliminary phytochemical screening [21]

1.*Carbohydrates (Molisch's test)*
To the test solution add few drops of alcoholic a-naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colour ring appears at the junction.
2.*Proteins (Trichloroacetic acid test)*
To the test solution add Trichloroacetic acid, precipitate is formed.
3.*Amino acids (Ninhydrine test)*
To the test solution add Ninhydrine solution, boil, violet colour indicates presence of amino acid.
4.*Alkaloids (Dragendorff,s reagent)*
Alkaloids give reddish brown precipitate with Dragendorff,s reagent (potassium bismuth iodide solution).
5.*Flavonoids (Shinoda test)*
To the test solution add few magnesium turnings and concentrated hydrochloride acid dropwish, pink scalet, crimson red or occasionally green to blue colour appears after few minutes.
6.*Steroids (Libermann-burchard test)*
Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids.
7.*Triterpenoids (salkowski test)*
Treat the extract with few drops of concentrated sulphuric acid, yellow coloured lower layer indicates presence of Triterpenoids.
8.*Glycosides*
Extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on water bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1 ml of fehling's solution A and B until it becomes alkaline (test with pH paper) and heat on a water bath for 2 minutes. The formation of red precipitate indicates the presence of glycosides.
9.*Cardiac glycoside (Legal's test)*
Treat the test solution with picric acid or sodium picrate, orange colour is formed.
10. *Tannins (Ferric chloride test)*
Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.
11. *Cholesterol*
Treat 2 ml of extract with 2ml of chloroform in a dry test tube and add 10 drops of acetic anhydride solution follow with 2 to 3 drops of concentrated sulphuric acid, red colour changes to blue or green colour indicates presence of cholesterols.

Statistical Analysis:
All the results were expressed as Mean ±S.E.M. of six animals in each group. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by student's t test. At 95% Confidence interval, p values < 0.001 were considered significant.

RESULTS AND DISCUSSION

The qualitative phytochemical investigation of aqueous extracts of flower of *Nerium olender* showed the presence of active chemical constituents such as Carbohydrates, Alkaloids, Flavonoids, Steroids, Glycosides, and Tannins. Absence of phytochemical such as Proteins, Amino acids, Triterpenoids and Cholesterol (Table 1).

Table-1: Preliminary phytochemical screening of Aqueous extract of *Nerium olender*.

S. No	TEST FOR	Aqueous extract for *Nerium olender* flower
1.	Carbohydrates	+
2.	Proteins and Amino acids	-
3.	Alkaloids	+
4.	Flavonoids	+
5.	Steroids	+
6.	Triterpenoids	-
7.	Glycosides	+
8.	Tannins	+
9.	Cholesterol	-

(+) = Present, (–) = Absent

The results of anthelmintic activity revealed that aqueous extracts of *Nerium olender* flower exhibit varying degree of activity like paralysis of worms followed by its death at all tested concentrations. From the above observations made the extract of *Nerium olender flower* was found to show potential anthelmintic activity when compared to standard drug in a dose dependent manor Table 2. Aqueous extract of *Nerium olender flower at a* concentration of 100mg/ml showed paralysis at 03.40 min and death of earthworm at 05.52 min which was comparable to standard Albendazole (Table 2). From the result, it is clear that aqueous extract of *Nerium olender* flower have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug.

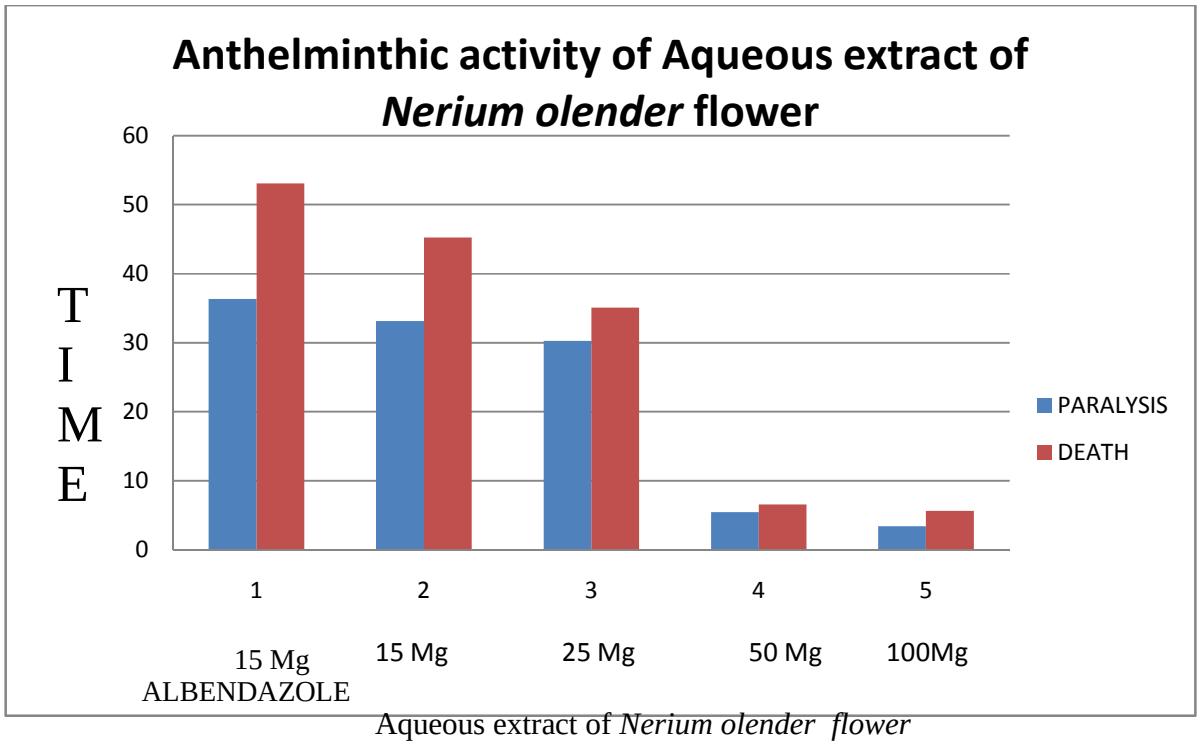

Figure 2-Anthelminthic activity of Aqueous extract of *Nerium olender* flower.

Table 2: Anthelmintic Potency of *Nerium olender* flower extract

Treatment	Group	Concentration (mg/ml)	Time of paralysis (min) (Mean±S.E.M)	Time of Death(min) (Mean±S.E.M)
Normal Saline (Control)	I	–	–	–
Albendazole	II	15	36.33±0.32	53.09±0.07
Aqueous extract of *Nerium olender* flower	III	15	33.11±0.82	45.29±0.35
	IV	25	30.26±0.18	35.09±0.91
	V	50	05.43±0.31	6.55±0.35
	VI	100	03.40±0.29	05.58±0.45

All values represent Mean ± SEM; n=6 in each group. All values are significantly different from reference standard (Albendazole). This activity was Concentration dependent. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms.

CONCLUSION

From the results it concludes that, aqueous extract of *Nerium olender flower* demonstrate to possess dose dependant anthelmintic activity when compared to Albendazole (Figure 1). The anthelmintic activity of Nerium olender flower was found to be inversely proportional to the time taken for paralysis and time of death of the worms. The active constituents responsible for anthelmintic activity are present in the aqueous extract of *Nerium olender.* The possible mechanism of the anthelminthics activity of Nerium olender *cannot* be explained on the basis of our present results. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic activity.

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