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In-vitro Anthelmintic Activity of *Oleandra musifolia* (Bl.) Presl. Against *Haemonchus contortus*

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ABSTRACT
Ethanolic, aqueous and Petroleum ether extracts from the whole fern, *Oleandra musifolia* (Bl.) Presl. (Family- Oleandraceae) was investigated for anthelmintic activity against *Haemonchus contortus*, with varied drug concentration (25, 50, and 100) mg/ml and the time taken for paralysis and death of the worm was determined. The *in-vitro* anthelmintic activity was compared with standard reference drug Albendazole and control distilled water. The result showed ethanolic extract performed well to express the anthelmintic efficacy than aqueous and petroleum ether extracts and the standard Albendazole. However efficacy is better and directly proportional to the increase in varied drug concentration. The efficacy is determined as lesser time taken to cause paralysis and death of *Haemonchus contortus* as compared to standard reference drug.

Keywords: Anthelmintic, *Oleandra musifolia* (Bl.) Presl, Fern, *Haemonchus contortus*.

INTRODUCTION

Parasitic disease mostly helminthiasis is the condition resulting from round worm infestations commonly *Haemonchus contortus* in small ruminants, is one of the major prevalent diseases in the world, particularly in the tropical countries, India. [1] The use of anthelmintics, notably the development of resistant in helminthes [2] to various anthelmintic compounds and classes, as well as chemical residue and toxicity problems create awareness towards ethnic medicine.

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In general, recognition of the antigenic complexity of development.

Consequently there is an urgent and ever present need to control infections caused by *H. contortus* in ruminants. The frequent use of anthelmintics over many years has inevitably led to the development of drug resistance to each class in parasitic nematodes. *H. contortus* has been documented to be resistant to all three broad spectrum families of anthelmintics *viz.*, benzimidazole, imidazothiazole and ivermectin. The emergence of resistance to anthelmintic drugs and the increased awareness of consumers about drug residues that potentially enter the food chain have stimulated investigation into alternative to commercially available anthelmintics such as medicinal plants. [3] For centuries, medicinal plants have been used to combat parasitism

and in many parts of the world are still used for this purpose.

The use of medicinal plants for the prevention and treatment of gastro-intestinal parasitism has its origin in ethno-veterinary medicine. The problems connected with the use of herbal medicine, the largest being the lack of scientific evaluation. The most effective approach to obtain such evaluation is the ethnobotanical approach, which assumes that indigenous uses of plant indicate the presence of biologically active compounds in the plants. Hence there is an increasing demand towards natural anthelmintics. [4]

Oleandra musifolia (Bl.) Presl. (Family: Oleandraceae) a creeping rhizome , 5–8 mm thick, white waxy in the older parts, often supported above the substrate by unbranched stilt-like roots, dorsiventrally flattened, (strongly compressed, 4-6 mm wide when dry), with up to 10 cm long, curved internodes (only occasionally straight and then often much longer) terminating in a cluster of a few short phyllopodia, usually less than 5-15 mm long, often hidden by the scales, rhizome often innovating just below this cluster, lateral branches usually basal on the internodes, in opposite pairs; all parts in cross-section without or with few sclerified strands, roots scattered, with long unbranched aerial parts. Scales persistently covering the rhizome, peltate, appressed, with dark center and lighter brown acumen and margin, margin ciliate especially when young and with sessile glands. [5] Fronds monomorphic, stipe 0.5–4 cm long, without dark colouration, with short, glandular hairs; lamina to 60 × 4.2 cm, linear, base cuneate to truncate or more or less rounded, apex acute to long-acuminate, texture thin-chartaceous, both surfaces with catenate, often glandular hairs 0.2–0.5 mm long; costa without dark colouration, on lower surface with inconspicuous, 1–3 mm long brown scales. Sori mostly in a single regular row close to the costa, sometimes more scattered over a 2–5 mm wide zone close to the costa or at a distance of up to 3 mm, indusium distinct, 1.5–2 mm wide, glabrous or glandular, sometimes setose. This medicinal plant is common in Western Ghats of India and is used as emmenagogue, against snake bite and as an anthelmintic. [6]

MATERIALS AND METHODS

The whole part of fern *Oleandra musifolia* (Bl.) Presl. were collected from Mudhumalai sanctuary, Nilgiri district, part of Western Ghats, Tamil Nadu and the herbarium was confirmed with the herbarium of Scott Christian College, Nagercoil.

Preparation of plant extracts

Fresh leaves, stems and roots were washed in running tap water and cut into small pieces and were shade dried and then in Hot air oven at 55-60°C. Dust was prepared by pulverizing the dried leaves, stems and roots with the help of mixer. A 25- mm, mesh diameter sieve was used to obtain fine dust and preserved them into airtight plastic container till their use in extract preparation. 10 gram of dust were taken in a 500 ml beaker and separately mixed with 100 ml ethanol. Then the mixture was stirred for 30 minutes by a magnetic stirrer (1000 rpm) and left stand for next 24 hours. The mixture was then filtered through Whatman filter paper, No 1. The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent from filtrate in a water bath at 50°C for ethanol up to final volume of 10 ml. [7] After the evaporation of solvent from filtrate, the condensed extracts were preserved in tightly corked-labelled bottle and stored in refrigerator until their screening for anthelmintic property. Similar procedure was adopted for the preparation of aqueous and petroleum ether extract as per. [8]

Worm collection and authentication

Adult live nematodes, *Haemonchus contortus* were collected from Perambur slaughter house, Chennai, from the g/I tracts (abomasm) of Sheeps. They were opened in a plastic bucket separately and the contents were washed up in tap water. The process was repeated for several times until the sediment becoming transparent. Then the adult g/I worms were collected with the help of a needle and placed in a petridish containing PBS (Phosphate Buffer Saline). Petridish containing the worms was kept in incubator at 38°C until required for experiment on the same day. *In-vitro* screening with ethanolic, aqueous and petroleum ether extracts of *Oleandra musifolia* (Bl.) Presl. was performed using *Haemonchus contortus.* [8] The ethanolic plant extracts were used at various concentrations *i.e.,* 25 mg/ml (2.5%), 50 mg/ml (5%) and100 mg/ml (10%), distilled water (control) and reference standard Albendazole (2.5%, 5% and 10%) using adult nematode worms (n=6) in petridish. Observations were made for time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour. Statistical analyses were carried out and the results were expressed as mean ± SEM. [9]

RESULTS AND DISCUSSION

From the observation made ethanolic extracts exhibited more potent activity than aqueous and petroleum ether extract against the nematode *Haemonchus contortus*. Evaluation of anthelmintic activity was compared with standard Albendazole. The ethanolic extract of *Oleandra musifolia* (Bl.) Presl. produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death (Table 1 and Figure 1). The aqueous and Petroleum ether extract showed significant activity. However in the present study, it was observed that ethanolic extract of *Oleandra musifolia* (Bl.) Presl. have exhibited positive and potent response than aqueous and Petroleum ether extract and the

standard Albendazole. The order of activity was ethanolic extract greater than aqueous extracts followed by petroleum ether, confirmed to the study of (Semwal & Farswan, 2012) in *Diplazium esculentum*. [8] The activity revealed concentration dependence nature of the different extracts.

Table 1: Anthelmintic activity of whole plant extract of *Oleandra musifolia* (Bl.) Presl.

Test Substance	Conc (mg/ml)	Time taken for paralysis (minutes)	Time taken for death (minutes)
Distilled Water (Control)	-	-	-
Albendazole (Standard)	25	22.167 ± 1.376	54.167 ± 1.046
	50	18.333 ± 1.054	35.167 ± 0.792
	100	17.333 ± 0.989	28.667 ± 0.667
Aqueous extract	25	44.000 ± 0.730	66.833 ± 0.872
	50	39.333 ± 0.494	55.167 ± 0.946
	100	33.167 ± 0.477	47.500 ± 0.671
Ethanolic extract	25	18.667 ± 0.715	45.833 ± 0.477
	50	15.833 ± 0.749	32.333 ± 0.667
	100	13.167 ± 1.195	24.167 ± 0.601
Petroleum ether extract	25	55.833 ± 0.792	80.333 ± 0.715
	50	47.167 ± 0.872	67.333 ± 0.494
	100	40.833 ± 0.654	54.500 ± 0.428

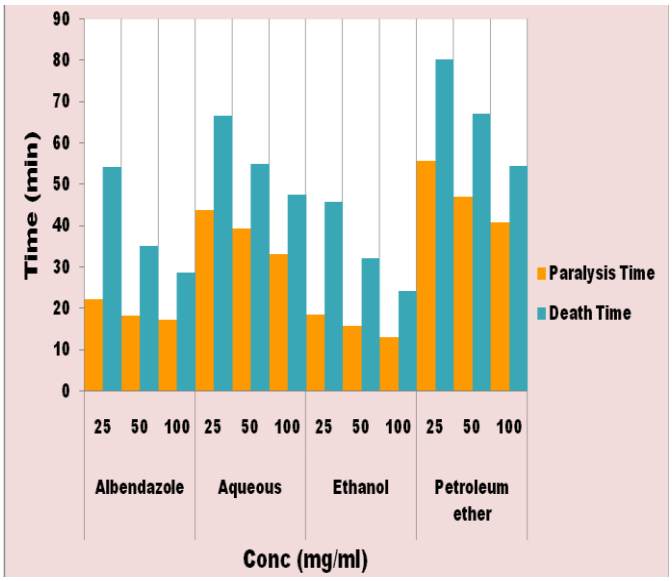

Fig. 1: *In-vitro* anthelmintic activity of *Oleandra musifolia* (Bl.) Presl.

It could be conclude that the ethanolic extract showed more potent anthelmintic activity. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of *Oleandra musifolia* (Bl.) Presl. as an anthelmintic drug.

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