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Anthelmintic activity of leaves extracts of *Olea europaea* on *Pheretima posthuma*

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ABSTRACT

Parasitic roundworms (nematodes) cause substantial morbidity and mortality in livestock animals globally and considerable productivity losses to farmers. The control of these nematodes has relied largely on the use of a limited number of anthelmintics. However, resistance to many of these anthelmintics is now widespread, and, therefore, there is a need to find new drugs to ensure sustained and effective treatment and control into the future. The present study was undertaken to evaluate the anthelmintic activity of crude aqueous, Petroleum ether, chloroform and methanol extract *Olea europaea* leaves using *Pheretima posthuma* as test worms. Single concentration (5%) of extracts was tested in the bioassay, which involved the determination of the time of paralysis (P) and time of death (D) of the worms. Piperazine citrate was included as a standard reference and distilled water as a control. The results of the present study indicated that *Olea europaea* leaves extracts were exhibited anthelmintic activity significantly when compared with the standard (Piperazine citrate) group. Further studies are in process to isolate the active principles responsible for the activity.

Keywords: *Olea europaea; anthelmintic; Pheretima posthuma.*

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INTRODUCTION

Olea europaea L., (Oleaceae) has been used widely in folk medicine in the north east of India and other Asian countries. The plant is widespread in the Arabian Peninsula, the Indian subcontinent and Asia and other tropical and subtropical parts of the world[1]. Several subspecies are recognized, one of which is the small fruited subspecies Africana (formerly Olea Africana). Although *O. europaea* is thought to be derived from subspecies Africa, in the early 80s the African wild olive was defined as *O. europaea*, subspecies Africana. Recently it was that from 120 plant species, mnquma was designated 'the most important plant' in use in the traditional medicine[2].

In traditional medicine, the plant is used as a diuretic, hypotensive, emollient, febrifuge and tonic, for urinary and bladder infections and for headaches. The hypotensive and hypoglycemic effects of olive leaves from *O. europaea* have been well documented[3].

Studies on the active principles of the olive leaf, the two Seco iridoids oleuropein, and oleoresin have been conducted for decades. It was reported that the bitter glycoside oleuropein had a hypotensive, coronary dilating and antiarrhythmic action. Recently, a bioassay-directed fractionation showed that another component of European olive leaf, beta-(3,4- dihydroxy phenyl) ethanol was a potent calcium antagonist. The isolate by fractionation from the olive leaf, Seco iridoid oleacein, was reported to have distinct angiotensin converting enzyme (ACE) inhibitory effect and antioxidant activity[4-5].

In the light of the above information, the present investigation was undertaken to evaluate the anthelmintic potential of *Olea europaea* leaves extract and is being reported here. Keeping these views in mind, the present study was planned to evaluate the anthelmintic activity of leaves extracts.

MATERIAL AND METHODS

Plant Material

Olea europaea leaves were collected from the forest of Tripura North, India in January 2015 and identified by a botanical survey of India (BSI) Hyderabad where a voucher specimen was deposited for reference.

Preparation of Extract

Shade-dried leaves powder was extracted with petroleum ether, chloroform, methanol (90%) and distilled water by soxhletion. The extract was concentrated by rotary vacuum evaporator. The dried extract was stored in airtight container in the refrigerator below 10°C. The extract was suspended in distilled water for experiments[6].

Worms Collection and Authentication

Adult earthworms *Pheretima posthuma* was used to evaluate anthelmintic activity in vitro. Earthworms were collected near the waterlogged areas of the rural area of Siddipet, Telangana state India. The average size of earthworm was 6-8 cm and was identified by a veterinary practitioner.

Preparation of test sample

Samples for the *in-vitro* study were prepared by dissolved 5 gm extract in 100 ml purified water to make 5 % solution of test extracts. 10 ml of the same solution was taken in each respective Petri dishes.

Anthelmintic assay

For the Anthelmintic activity of leaves extracts of *Olea europaea* leaves, Indian adult earthworms (*Pheretima posthuma*) of 3-5 cm in length and 0.1 – 0.2 cm in width were used. The earthworms were divided into six groups containing five earthworms in each group. All the extracts were freshly prepared in 5% concentration before starting the experiments. Different extracts were poured in different Petri dishes. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows: distilled water (10 ml), piperazine citrate (10 mg/ml), petroleum ether (50 mg/ml), chloroform extract (50 mg/ml), methanol extract (50 mg/ml), and aqueous extract (50 mg/ml). Observation were made for the time taken to paralysis (paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously) and Death (Death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0C). Piperazine citrate was used as reference standard while distilled water as a control[7-8].

RESULTS AND DISCUSSION

Continued reliance on mass drug administration with a limited number of synthetic anthelmintics has the potential to place heavy selection pressure on drug-resistant parasites, and widespread anthelmintic drug resistance is already a serious problem in many livestock production systems. The use of natural dietary compounds has the potential to be a complementary control option which may reduce this reliance on drug treatment, and slow the development of resistance. Here we have carried out a comprehensive *in vitro* assessment of the effects of *Olea europaea* different extract on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[9-11]. *Posthuma* worms are easily available and used as a suitable model for screening of anthelmintic drug was advocate earlier.[12-14].

In the present study, *Olea europaea* leaves fresh leaves extracts were exhibited anthelmintic activity significantly when compared with standard group. Whereas, in control group, worms were observed for 24 hours and no paralysis or death was found during that period. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis[15-16].

The leaves extract of Olea europaea not only demonstrated paralysis but also causes the death of worms especially at a higher concentration of 50 mg/ml, in a shorter time as compared to reference drug piperazine citrate. The results were showed on the Table No. 1 and Figure 1

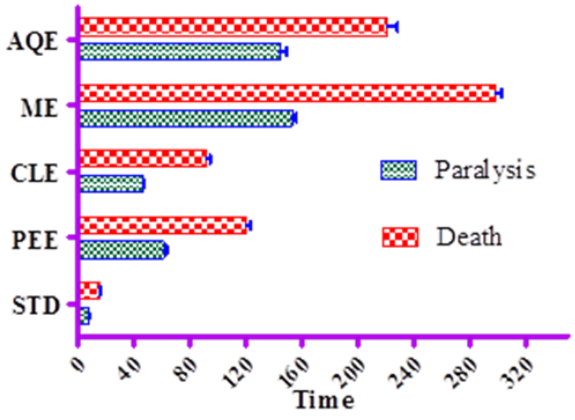

Figure 1: Anthelmintic activity of *Olea europaea* leaves extracts

CONCLUSION

On the basis of these investigations, we may partially conclude that *Olea europaea* leaves could be a potent anthelmintic agent for next generation. We propose that future work should focus on attempting to fractionate extract PEE and CLE in order to identify and characterize the constituent(s) that are active against helminthics infection, and then to explore which biological pathways are affected by these components/fractions.

Table 1: Anthelmintic activity of *Olea europaea* leaves extracts

S. No.	Treatment	Concentration (in mg / ml)	Time is taken for paralysis and death of worms in minutes	
			Paralysis	Death
1	Control	10 ml	---	---
2	Piperazine citrate	10	007.7 ± 0.270	015.4± 0.546
3	Petroleum ether extract	50	061.24 ± 1.97	120.51 ± 2.806
4	Chloroform extract	50	046.44± 1.240	092.207 ± 2.337
5	Methanol extract	50	153.56 ± 3.048	298.13 ± 4.642
6	Aqueous extract	50	144.24 ± 4.681	220.68± 7.501

Values are mean ± SEM (n=5), group, one way ANOVA test.

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