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 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.

Keywords: Olea europaea; anthelmintic; Pheretima posthuma.
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 soxhletation. 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°C)). 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 europa* 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

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 helminths 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                        | 0.07 ± 0.270            | 0.15 ± 0.546                   |
| 3      | Petroleum ether extract    | 50                        | 0.61 ± 1.97             | 12.50 ± 2.086                  |
| 4      | Chloroform extract         | 50                        | 0.46 ± 1.240            | 0.92 ± 2.337                   |
| 5      | Methanol extract           | 50                        | 15.36 ± 3.048           | 29.13 ± 4.642                  |
| 6      | Aqueous extract            | 50                        | 14.24 ± 4.681           | 22.60 ± 7.501                  |

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

REFERENCES

1. Asolkar LV, Kakker KK, Chahre OJ. Glossary of Indian medicinal plants, National Institute of Science and Communication, New Delhi, 2000, p. 119
2. Capretti G, Bonaconza E. Effects of infusions or decoctions of olive leaves (*O. europaea*) on some constant physical conditions of blood and components of metabolism. Giornale Clinica Medicina. 1949; 30: 630-642.
3. Casanovas M, Florido F, Saenz de San Pedro B, Gonzales P, Martínez-Alzamora F, Maranon F, Fernandez-Caldas E. Sensitization to *O. europaea*: geographical differences and discrepancies. Allergologia Immunopathol (Madr). 1997; 25(4): 159-166.
4. Cherif S, Rahal N, Haouala M, Hizaoui B, Darougth F, Gueddiche M, Kallel Z, Balansard G, Boukef K. A clinical trial of a titrated *Olea* extract in the treatment of essential arterial hypertension. J Pharm Belq. 1996; 51(2): 69-71.
5. Cortesi N, Mosconi C, Fedeli E. High performance liquid chromatography in the analysis of *O. europaea* leaf extracts. Chemical Abstracts. 1985; 10: 859-871.
6. Fehri B, Aiache JM, Memmi A, Korbi S, Yacoubi MT, Mrad S, Lamaison JL. Hypotension, hypoglycemia and hypouricemia recorded after repeated administration of aqueous leaf extract of *O. europaea* L. J Pharm Belq. 1994; 49: 101-108.
7. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In-vitro anthelmintic properties of *Buchholzia coleaceae* and *Gynandropsis gynandra* extract. Pharm Biol. 2001; 39(3): 217-20.
8. Manoj Aswar, Urmila Aswar, Bhagyashri Watkar, Meenakshi Vyas, Akshaya Wagh, Kishore N. Gujar. Anthelmintic activity of *Ficus benghalensis*. Int J Green Pharm. 2008; 2(3): 170-172.
9. Thorn GW, Adams RD, Braunwald E, Isselbacher KJ, Petersdorf RG. Harrissons Principles of Internal Medicine, McGraw Hill Co., New York, 1997, p. 1088.

10. Vigar Z. Atlas of Medical Parasitology, P.G. Publishing House, Singapore, 1984, p. 216.
11. Dash GK, Mishra B, Panda A, Patro P, Ganpaty S. Anthelmintic activity of *Evolvulus nummularis*. Ind J Nat Prod. 2003; 28: 19-24.
12. Tambe VD, Nirmal SA, Jadhav RS, Ghogare PB, Bhalke RD, Girme AS, Bhamber RS. Anthelmintic activity of *Wedelia trilobata* leaves. Ind J Nat Prod. 2006; 22(3): 27-29.
13. Mali RG, Mahajan SG, Mehta AA. *In-vitro* anthelmintic activity of stem bark of *Mimusops elengi* Linn. Pharmacogn Mag. 2007; 3(10): 73-76.
14. Dash GK, Suresh P, Sahu SK, Kar DM, Ganpaty S, Panda SB. Evaluation of *Evolvulus alsinoides* Linn. For anthelmintic and antimicrobial activities. J Nat Rem. 2002; 2(2): 182-85.
15. Szewezuk VD, Mongelli ER, Pomillo AB. Antiparasitic activity of *Melia azadirach* growing in Argentina. Mole Med Chem. 2003; 1: 54-57.
16. Shivkar YM, Kumar VL. Anthelmintic activity of latex of *Calotropis procera*. Pharm Biol. 2003; 41(4): 263-65.