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In-vitro anthelmintic activity of *Rhizophora mucronata* leaves extract against *Pheritima posthuma*

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Abstract
In this present experiment we had studied about to evaluate *in vitro* anthelmintic activity of *Rhizophora mucronata* by using leaves extract. Anthelmintic activity was tested against Indian earthworm *Pheretima posthuma*. Extraction of *Rhizophora mucronata* demonstrated potent anthelmintic activity tested against Indian earthworm *Pheretima posthuma*. The dose-dependent anthelmintic efficacy of the fractions was quite similar to that of piperazine citrate The result obtained in the study led to the conclude that leaves of the mangrove plant, high level of polyphenolics and show significant anthelmintic activity.

Keywords: *Rhizophora mucronata*, anthelmintic activity, *Pheretima posthumous*

Introduction
Rhizophora mucronata (mangroves) are prominent halophytic inhabitants of intertidal zone found in tropical and subtropical climates thriving under varying degrees of salinity, ranging from fresh to levels greater than that of seawater [1]. Salinity imposes quite a few stumbling blocks in overall plant cellular metabolic processes which includes water deficit caused by salt induced osmotic stress and the toxic effects due to ion excess. These salt-tolerant plants (mangroves) have advanced mechanisms to cope with the harmful after effects of salinity stress. Despite the fact that stress tolerance mechanisms of plants materialized to be composite and divergent, various lines of mechanisms have been proposed for their salt tolerance properties: osmotic potential across the cell membrane is balanced by the accumulation of low molecular weight osmolytes, such as glycine betaine, sugar alcohols and proline [2-4] and stress induced damages get repaired by the production of stress-inducible proteins and other biomolecules, of which vivid physiological function is yet to become apparent [5-9]. The plant-cell membrane itself is a basic and potential barrier to a number of external factors in addition to these metabolic shifts to overcome environmental difficulties. Here comes the paramount part of lipids in cell membranes for adapting plants to environmental stresses. Salt stress prompted alterations in plant lipid bilayer composition and membrane permeability due to salt stress were reported earlier by various authors [10-12]. Such modifications of the lipid bilayer result in transmuted membrane fluidity and H^+-ATPase activity, influencing the passive influx of potentially toxic ions such as Na^+ and Cl^- [10, 13]. Plasma membrane permeability is an instinctive property of the lipid composition and the lipid-protein interaction [13]. In spite of this, few studies have focused on the lipid composition of mangrove plants. It is important to study the salt tolerance mechanism of individual genera or species because mangrove trees have evolved their own peculiar mechanism to adapt to specific environment and shows complexity and discrepancy between species. Although mangroves are comprised of a great diversity of plants, the genus Rhizophora is one of the prevalent representatives of the plant groups in India [14]. *Rhizophora mucronata* is a small to medium size evergreen tree growing to a height of about 20 to 25 metres (66 to 82 ft) on the banks of rivers. On the fringes of the sea 10 or 15 metres (33 or 49 ft) is a more typical height. The tallest trees are closest to the water and shorter trees are further inland. The tree has a large number of aerial stilt roots buttressing the trunk. The leaves are elliptical and usually about 12 centimeters (4.7 in) long and 6 centimeters (2.4 in) wide. They have elongated tips but these often break off. There are corky warts on the pale undersides of the leaves. The flowers develop in axillary clusters on the twigs. Each has a hard cream-coloured calyx with four sepals and four white, hairy petals. The seeds are viviparous and start to develop whilst still attached to the tree. [15] The root begins to elongate and may reach a length of a metre (yard) or more. The propagule then becomes detached from the branch when sufficiently well developed to root in the mud below. [16]

Fig 1: Plant of *Rhizophora mucronata*

Materials and Methods
Plant materials and preparation of extracts
The collected *Rhizophora mucronata* leaves were processed on the same day itself. The leaves were washed thoroughly with distilled water and freeze dried. The dried samples were

ground to powder and stored in air tight until further analysis. The powdered leaf material was soaked in the different solvents of varying polarity such as methanol, acetone and at room temperature for 24 h with mass to volume ratio of 1:40 (g/ml). The solvents were filtered through Whatmans No. 1 filter paper to remove the solid particles. The filtered solvents were evaporated to dryness under vacuum on a rotary evaporator at 40°C. Water extract of R. mucronata was prepared as above by soaking dried powder in distilled water and stirred using a magnetic stirrer at a low speed for 24h.

Anthelmintic Activity

Anatomical and physiological characteristic of Indian earth worm resemblance with the intestinal round worm parasite of human being, therefore Pheretima posthuma have taken in this study to assess anthelmintic activity of *R. mucronata*. Indian earth worms are divided into three groups each containing six earthworms approximately of equal size in following manner:

- Group I: Control (2% Tween 80 in normal saline)
- Group II: standard (15, 30 and 45 mg/ml)
- Group III: Plant extract (15, 30 and 45 mg/ml)

Fifty milliliters of respective drug solutions were taken in petri dishes and the earthworms were released in to the solution. Earth worms were monitored carefully and observations were made for the time taken to paralyze and death of individual worms. Time taken to till paralysis was recorded when no movement could be observed except when the worms were shaken vigorously. Times taken for death of worms were noted after ascertaining that the worms lost their motility completely with fading of their body colour. To confirm, the death worms were shaken vigorously or dipped in warm water at 50 °C but no movement was observed.

Results and Discussion

Table.1: In Vitro Anthelmentic Effect of *Rhizophora mucronata* Leaves Extract Against *Pheretima posthuma*

Groups	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Control	----	----	----
Standard	15	29.67±0.31	61.31±1.17
	30	21.19±0.51	47.15±1.71
	45	19.11±0.77	21.13±1.43
Plant extracts	15	45.87±0.29	118.34±1.41
	30	30.28±0.37	106.21±1.88
	45	21.17±0.42	76.47±0.47

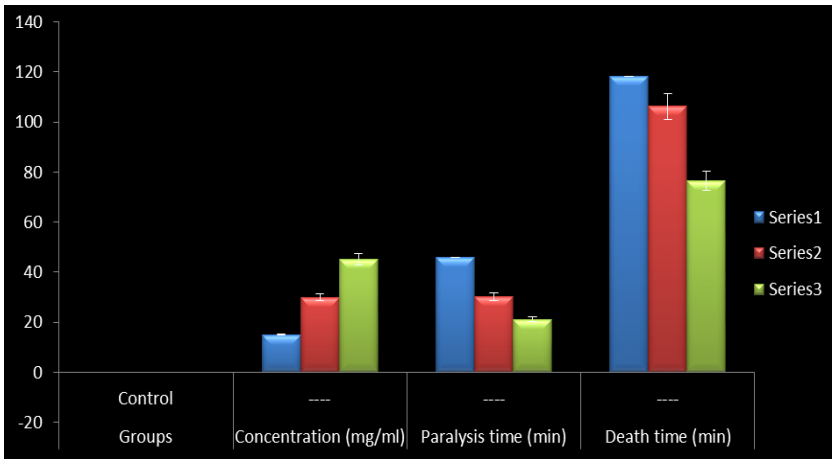

Fig 2: Graph for Standard

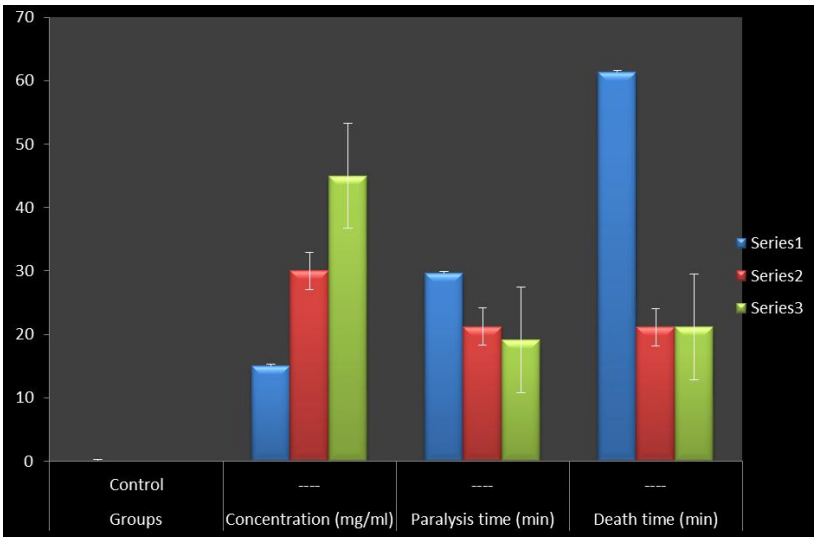

Fig 3: Graph for Plant Extrac

Anthelmintic activity of leaf extract of *Rhizophora mucronata* was performed against Indian earthworm *Pheretima posthuma*. *R. mucronata* extract produced moderate activity. At 15, 30 and 45 mg/ml concentration, extract produced paralysis in worms after 45.87±0.29, 30.28±0.37 and 21.17±0.42min, while at same concentration after 118.34±1.41, 106.21±1.88 and 76.47±0.47min produced death in earthworms respectively. Standard drug piperazine citrate at a 15 and 30 mg/ml, 45 mg/ml concentration, showed the potent activity which was evident by the quick paralysis time 29.67±0.31, 21.19±0.51 and 19.11±0.77respectively and death time 61.31±1.17, 47.15±1.71 and 21.13±1.43min respectively. The paralysis and death times of the extract, fractions and standard drug are given in Table 1. Depicts the *Pheretima posthuma* state with control, extract and piperazine citrate.

Conclusion

According to the above study it was concluded that the Extraction of *Rhizophora mucronata* demonstrated potent anthelmintic activity tested against Indian earthworm *Pheretima posthuma* but it did not give clear inference at that stage and hence we considered the work for further extensive research.

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