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In-vitro anthelmintic activity of *Rhizophora mucronata* leaf extract

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ABSTRACT

To evaluate invitro anthelmintic activity of Rhizophora mucronata anthelmintic activity tested against Indian earthworm Pheretima posthuma In this present study, Extraction of R. 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 conclusion that leaves of the mangrove plant, high level of polyphenolics and show significant anthelmintic activity

Keywords: Anthelmintic effect, *R.mucronata, Pheretima posthumous*

INTRODUCTION

Many helminthes infection are considered as accidental infections from other species that are normal hosts or perhaps common in some communities. Most of the GI helminthes infections are light and asymptomatic; they do not cause significant morbidity and mortality, but have a prominent impact on nutrition, pregnancy outcome, growth, physical fitness, cognitive functions and anaemia in infants, children and adults. Many factors like social, behavioral and genetic determine the susceptibility of infections in an individual. Age also considered as key factor for GI Helminthes infection vary between species; there is an increase in hookworm intensity with age, but high intensity in childhood for Ascaris and Trichuris infections[1].the protective immune response against many helminth parasites generally referred as TH_2 (T helper 2) response. TH_2 cells orchestrate the immune response mainly through the production of several cytokines in the lymph nodes and periphery. TH_2-type responses are naturally considered by the increase levels of interleukin-4 IL-4) and cytokines like IL-5, IL-9, IL-13 and IL-21, activation and expansion of $CD4^+$.TH_2 cells, plasma cells secreting immunoglobulin E (IgE), eosinophils, mast cells and basophils, all of which can generate several types of TH_2-type cytokine. IL-5 triggers eosinophilia, and in combination with IL-4, IL-9, and IL-13, and crosslinking of FcRIs (high-affinity Fc receptors for IgE) results improved mast-cell and basophil development and release of mediators. IL-4 and IL-13 causes amplified smooth muscle cell contractility, increased intestinal permeability, increased goblet-cell mucous secretion and enhance mast-cell-derived mediators. Together, these effects can cause to the „weep and sweep" response to intestinal helminths. IL‑4 can induce class switching in B cells in conjunction with other signals that leads to IgE production. IL-4, IL-13 and IL-21 can trigger the development of alternatively activated macrophages, leading to up-regulation of arg.inase-1 expression, though in some cases this might lead to fibrosis, as in chronic schistosomiasis. IL-17 linked cytokine IL-25 is also connected with the TH2-type response and can endorse TH_2-cell differentiation and nematode parasite expulsion, though the nature of IL-25 is remains unclear. In comparison, interferon-γ dominant TH1-type responses are classically evoked by microbial (bacteria and viruses) infections, and are related with increase in TH1 cells, cytotoxic CD8+T cells,

neutrophils and macrophages count. Initially IL-10 was characterized as a TH2-type cytokine, but resent researchers suggested that this cytokine is also produced by TH1 cells and regulatory T cells in vivo, and can down- regulate both TH1-type and TH2-type responses. A number of cytokines like IL-4, IL- 13, IL-21 and IL-25 that are preferentially expressed during the TH2-type response can also down regulate TH1-type and TH17-type responses and their associated inflammation [2].

Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods includes proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemo pharmacological investigations, toxicological and clinical studies, standardization and use of active moiety as the lead molecule for drug design. Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell [3][4], Even with the advent of modern or allopathic medicine [5], have noted that a number of important modern drugs have been derived from plants used by indigenous people.Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources [5], Plants are used medicinally in different countries and are a source of many potent and powerful drugs [6] [7].

MATERIALS AND METHODS

Chemicals and Reagents:
All chemicals and reagents used in the experiments were of analytical grade and obtained from Merck (Mumbai, India)

Plant materials and preparation of extracts
Rhizophora mucronata a mangrove plant was collected from the inter tidal area of the Neil Island, Port Blair, A& N Islands, India. The plant was botanically identified and authenticated by Mr. Rajanala VenuMadhav, Department of Botany, S.S.N College, Narasaraopet, and Guntur. The collected mangrove 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 at – 20 °C 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 Whatman 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.

METHOD
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 (3% Tween 80 in normal saline)
Group II : standard (10, 20 and 50 mg/ml)
Group III : Plant extract (10, 20 and 50 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

ANTHELMINTIC ACTIVITY OF *R. MUCRONATA*

TABLE.1: *IN VITRO* ANTHELMINTIC EFFECT OF *R. MUCRONATA* LEAVES EXTRACT AGAINST *PHERITIMA POSTHUMA*

Groups	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Control	----	----	----
Standard	10	30.13±0.82	57.18±1.32
	20	23.03±0.67	45.15±1.41
	50	17.08±0.98	20.12±1.76
Plant extracts	10	42.67±0.32	120.34±1.32
	20	31.23±0.29	108.21±1.21
	50	22.12±0.42	80.47±0.32

Fig no: 1 *: In vitro* anthelmintic effect of *R. Mucronata* leaves extract against *Pheritima posthuma*

Figure 1.1: Study of anthelmintic activity, control group

Figure 1.2: Study of anthelmintic activity, piperazine citrate treated group

Figure 1.3: Study of anthelmintic activity, extract treated group

Anthelmintic activity of leaf extract of *R. mucronata* was performed against Indian earthworm *Pheretima posthuma. R. mucronata* extract produced moderate activity. At 10, 20 and 50 mg/ml concentration, extract produced paralysis in worms after 42.67±0.32and 31.23±0.29 and22.12±0.42 min, while at same concentration after 120.34±1.32and 108.21±1.21 and 80.47±0.32 min produced death in earthworms respectively. Standard drug piperazine citrate at a 10 and 20 mg/ml,50 mg/ml concentration, showed the potent activity which was evident by the quick paralysis time 30.13±0.82, 23.03±0.67and 17.08±0.98 respectively) and death time 57.18±1.32, 45.15±1.41and 20.12±1.76min respectively). The paralysis and death times of the extract, fractions and standard drug are given in Table 2. Fig 2 depicts the *Pheretima posthuma* state with control, extract and piperazine citrate.

CONCLUSION

From our present study it is concluded that the Extraction of *R. mucronata* demonstrated potent anthelmintic activity tested against Indian earthworm *Pheretima posthuma*.However no clear inference can be drawn at this stage and hence we consider the work for further extensive research.

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