ANTHELMINTIC ACTIVITY OF FRUIT EXTRACTS OF *FICUS GLOMERATA* ROXB.

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Summary

The anthelmintic activity of fruit extracts of an Indian Medicinal plant *Ficus glomerata* Roxb. was evaluated on earthworms of *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. The potency of the various concentrations of extracts was evaluated by time taken for paralysis and death of earthworms after treatment with the test drugs and compared with the reference drug piperazine citrate (15 mg/ml). The petroleum ether extract and acetone extract of *Ficus glomerata* fruits at higher doses of 50 mg/ml has shown significant activity against helminthes which was comparable to the reference drug. The results suggest that fruits of *Ficus glomerata* possess anthelmintic activity and the plant is worthwhile for further investigation to isolate and identify the constituents responsible for the activity.

Keywords: *Ficus glomerata*, Anthelmintic, *Pheretima posthuma*, Piperazine citrate, Fruit extract.

Introduction

The increasing prevalence of helminth parasites that are resistant to conventional anthelmintics (1) has been the spur for exploring alternative approaches to parasite control. Understanding the nature of the bioactive components, their mode of action and their targets within the parasites are all important in the process leading to practical application. Bioactive plants may contain large numbers of plant secondary metabolites that may act singly or in combination to produce direct (2) and/or indirect effects on parasites in the alimentary tract leading to reduced parasite survival, growth and fecundity. They can also provide improved protein availability in the host and/or have direct or indirect effects on mineral or trace element status. Both of these changes to host nutrition can lead to reductions in parasite establishment, burden fecundity through improvements in host immuno regulatory capacity.

There are number of extracts and active biochemical compounds from plants act against parasites including essential oils, proteolytic enzymes, lectins and polyphenolics such as the tannins (3). *Ficus glomerata* Roxb. belongs to the family Moraceae is a large deciduous tree distributed all over India in evergreen forests, moist localities and banks of streams. It is commonly known as Cluster fig in English, Gular in Hindi and as Udumbara in Sanskrit (3-6). The root is used in dysentery, diabetes, applied in mumps, other inflammatory and glandular enlargements. The bark is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccough, leprosy, dysentery and piles. The leaves are good wash for wounds and ulcers. Tender fruits are astringent, stomachic, and useful in treatment of leucorrhoea, blood disorder, fatigue, and carminative. Latex is aphrodisiac and administered in hemorrhoids, diarrhoea, boils, vaginal disorders and toothache (4, 7, 8). The present study was stemmed based on the reports that the fruit extract of *Ficus racemosa* exhibited prominent antifilarial activity against the worm *Setaria cervi in vitro* (13) and the aqueous bark extract of *Ficus glomerata* shown to possess anthelmintic activity by using adult earthworm *Pheretima posthuma*. However, there are no reports available on anthelmintic activity on the fruit extract against the worm *Pheretima posthuma*. Hence, the present study was undertaken to investigate the anthelmintic activity of fruit extracts of *Ficus glomerata* Roxb.

Materials and Methods

Plant material

The fruits of *Ficus glomerata* Roxb. (Moraceae) were collected in the first week of March from fruiting trees in places around Bajpe, Mangalore, Karnataka, India. They were washed, air dried for a week at 35-40˚C, powdered and stored in room temperature in a closed container for further experimental use.

Extract preparation

The powdered drug was repeatedly extracted in a soxhlet apparatus using solvents of increasing polarity with petroleum ether, benzene, chloroform, acetone, ethanol and distilled water and refluxed for 48 hours with each solvent. The extracts were collected and concentrated by evaporation. The extract is dried *in vacuo* and used for subsequent experiments.

Worm collection

The anthelmintic activity was evaluated on adult earthworm of *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (9-11).Indian earthworm *Pheretima posthuma* (Annelida, Megacolecide) was collected from the moist soil of water-logged areas from Karambar, Karnataka, India. The collected worms were washed with normal saline to remove all faecal matter and worms of almost equal size (around 6 cm) were grouped into seven of six each (12,13).

Evaluation of anthelmintic activity

Petroleum ether, benzene, Chloroform, acetone, ethanol and aqueous extracts were evaluated at the concentration of 10, 20 and 50 mg/ml and the reference standard (piperazine citrate) was tested at the concentration of 15 mg/ml (12-16). Twenty groups of *Pheretima posthuma* were placed in petridishes each containing six earthworms and each group was treated with the following concentration of drug in normal saline containing 1% tween 80: Group 1 : Piperazine citrate (15 mg/ml), Group 2: Petroleum ether extract (10 mg/ml), Group 3: Petroleum ether extract (20 mg/ml), Group 4: Petroleum ether extract (50 mg/ml), Group 5: Benzene extract (10 mg/ml), Group 6: Benzeneextract (20 mg/ml), Group 7: Benzene extract (50 mg/ml), Group 8: Chloroform extract (10 mg/ml), Group 9: Chloroform extract (20 mg/ml), Group 10: Chloroform extract (50 mg/ml), Group 11: Acetone extract (10 mg/ml), Group 12: Acetone extract (20 mg/ml), Group 13: Acetone extract (50 mg/ml), Group 14: Ethanol extract (10 mg/ml), Group 15: Ethanol extract (20 mg/ml), Group 16: Ethanol extract (50 mg/ml) Group 17: Aqueous extract (10 mg/ml), Group 18: Aqueous extract (20 mg/ml), Group 19: Aqueous extract (50 mg/ml) and Group 20: Control (1% tween 80 in normal saline).

Observations were made for time taken for paralysis and/or death of individual worms' up to 4 hours of test period. All the petridishes were placed at room temperature. The time of paralysis (P) was noted when no movement of any sort could be observed except when shaken vigorously. Death time (D) was recorded when there was no movement observed after vigorous shaking not even when they were dipped in warm water (50^{o}C). The mean time taken for paralysis and death was calculated.

Statistical Analysis

Data was analyzed statistically by one way ANOVA followed by post hoc Scheffe's test using the SPSS software package.

Results and Discussion

Helminthic infections of the gastrointestinal tract of human beings have been recognized to have adverse effects on health standards with a consequent lowering of resistance to other diseases. In search of compounds with anthelmintic activity, a number of substances were screened using different species of worms, for example, earthworms, *Ascaris*, *Nippostrongylus* and *Heterakis*. Of all these species, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro because they resemble intestinal "worms" in their reaction to anthelmintics and are easily available. Adult Indian earthworm, *Pheretima posthuma* has been used as test worm in most of the anthelmintic screenings, as it shows anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as anthelmintic.

After a brief stimulant effect, earthworms lost their motility on exposure to crude extracts of fruits of *Ficus glomerata*. Each extract produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. Petroleum ether and acetone extracts at the dose of 50 mg/ml showed significant wormicidal activity and was comparable with the standard drug piperazine acetate (Table 1). Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can

cause paralysis. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation and leading to paralysis.

Piperazine citrate causes flaccid paralysis by increasing chloride ion conductance of worm muscle membrane and produces hyper polarization resulting in the blockade of neuromuscular transmission and reduces excitability that leads to muscle relaxation. The anthelmintic activity of the roots may be attributed to the same mechanism. In the present investigation, petroleum ether and acetone extract showed significant activity. But further studies are necessary to confirm the safety and efficacy of crude extracts following internal needs to be ascertained.

Table 1

Test substance	Concentration (mg/ml)	Time taken (in minutes)	
		Paralysis (P)	Death (D)
Piperazine citrate	15	12.0 ± 0.8	23.66 ± 0.3
Petroleum ether extract	10	40.33 ± 0.2	50.66 ± 0.3
	20	25.66 ± 0.3	36.33 ± 0.3
	50	12.33 ± 0.2*	22.0 ± 0.4*
Benzene extract	10	116.66 ± 0.33	128.33 ± 0.21
	20	84.16 ± 0.5	96.33 ± 0.2
	50	47.16 ± 0.4	62.5 ± 0.2
Chloroform extract	10	155.66 ± 0.33	170.66 ± 0.33
	20	116.5 ± 0.22	131.5 ± 0.22
	50	57.0 ± 0.4	72.0 ± 0.4
Acetone extract	10	50.33 ± 0.2	60.83 ± 0.3
	20	33.5 ± 0.3	42.0 ± 0.4
	50	15.0 ± 0.3*	25.5 ± 0.3*
Ethanol extract	10	140.2 ± 0.31	181.66 ± 0.91
	20	48.16 ± 1.1	72.5 ± 0.8
	50	25.83 ± 0.3	42.33 ± 0.9
Aqueous extract	10	160.66 ± 0.21	178.83 ± 0.40
	20	120.5 ± 0.34	132.66 ± 0.49
	50	81.5 ± 0.3	97.0 ± 0.4
Control	-	-	-

Results expressed as Mean ± S.E.M; (n=6); *Significant at $p<0.05$; P value was calculated by comparing with control by one-way ANOVA followed by post Scheffe's method.

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