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In Vitro evaluation of anthelmintic activity of leaves extract of *Flacourtia jangomas*

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Abstract
The current study was carried out using the leaves of *Flacourtia jangomas*. The goal of this study was to check how well the water and alcohol extracts from the leaves of *Flacourtia jangomas* work against Indian earthworms called Perionyx posthuma. Piperazine citrate was used as the standard reference. The time it took to make the worms paralyzed and the time it took for them to die after being treated with the extracts was recorded, showing that the alcoholic extract worked better when used at higher concentrations.

Keywords: *Flacourtia jangomas*, anthelmintics, perionyx posthuma

Introduction

Helminthes are big, multi-celled creatures that don't have a backbone. They have complex body systems and usually have long, flat, or round shapes. Most of these different organisms are mainly active eaters and can be seen with the naked eye when they are adults [1].

There are two big groups of worms called nematodes and platyhelminths. Nematodes are also called roundworms and include soil-transmitted helminths as well as filarial worms that cause diseases like lymphatic filariasis and onchocerciasis. Another group of worms is called platyhelminths, which are flatworms and include flukes, schistosomes, and tapeworms such as the pork tapeworm that leads to cysticercosis. Flukes are a type of trematode, and tapeworms are a type of cestode [2, 3].

Soil-transmitted helminthiasis is a roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworm (*Ancylostomaduodenale* and *Necatoramericanus*) [4, 5, 6].

Soil-transmitted helminths enter the human body when people come into contact with soil that has eggs from A. lumbricoides and T. trichiura. Some types of worms can go through the skin directly, like hookworm larvae [6, 7].

Most of the people who have helminthiasis come from developing countries, particularly from rural regions where there is not enough cleanliness and proper hygiene practices. Most are also preschool and school-aged. In most situations, the way a person shows symptoms depends on how much of the worm infection they have, because some people might only have a light infection.

The main factors that increase the risk of getting helminthiasis include living in rural areas, having a low income, poor sanitation, not having access to clean water, not keeping good personal hygiene, not cutting nails regularly, living in crowded spaces, not having enough education, not being able to get medical care, and poor housing conditions. Research in the USA shows that about 1.3 to 2.8 million people have signs of Toxocara infection, 4 million have infections from soil-transmitted worms, between 41,400 and 169,000 have cysticercosis, and around 8,000 have schistosomiasis [8].

Helminths get passed on to the final host through different methods. The most common way people get infected is by eating vegetables that have germs, drinking water that isn't clean, or eating raw or not fully cooked meat. Certain types of worms can be recognized by looking at their eggs under a microscope, which are found in stool samples. More advanced tests like serological tests, antigen tests, and molecular testing are also available [9].

Anthelmintic is the term used to describe a drug used to treat infections of animals with parasitic worms. This includes both flat worms, e.g., flukes (trematodes) and tapeworms (cestodes) as well as round worms (nematodes). The parasites are of huge importance for human tropical medicine and for veterinary medicine [10].

Requirements: Collection of plant leaves

For this study, the leaves of *Flacourtia jangomas* were gathered from Kottayilkovilakam road in Chendamangalam, Ernakulam district. The plant was identified and authenticated by Ms. I.K Nishitha, specimen accession numbers DSAH002459, DSAH002460, DSAH002461. The leaves were dried in the shade and stored at room temperature. They were then crushed into a fine powder and sifted through a sieve labeled number 44. The powder needs to be stored in a container that closes tightly.

Choice of worms

Indian earthworms called Perionyx posthuma are used to test how well anthelmintic substances work. Earthworms are gathered from wet soil and rinsed with distilled water to get rid of all extra dirt. They share similar physical features with human intestinal worms.

Collection of earthworms

Indian earthworm species was collected and its species identified from Kerala Agricultural University, Vellanikkara Thrissur. The collected earthworm species was identified by Smt. Nimila P J, HOD of Zoology Department.

Management of piperazine citrate

Standard drug Piperazine citrate at 25mg/ml concentration is prepared, to check how effective it was against worms.

Administration of extract

The extract is given to the earthworms by putting it into petri dishes with Perionyx posthuma. The extract is made at

three different strengths—25mg/mL, 50mg/mL, and 100mg/mL—using water liquid base. Three earthworms are put into each petri dish with the extract solution. This method helps check how well the FJ leaf extract works against worms**.**

Experimental design

The Perionyx posthuma were collected and first cleaned with normal saline (NS 0.9%w/v). Eight Petri dishes were cleaned using distilled water and then sterilized with hot water. One Petri dish was used as a control, and another Petri dish was placed with the standard of piperazine citrate, along with three Perionyx posthuma. The other six petri plates have alcoholic and aqueous leaf extracts of FJ, with each type placed in three petri dishes at different concentrations. The first three petri dishes had an aqueous leaf extract of FJ, labeled as A1 (25mg/mL), A2 (50mg/mL), and A3 (100mg/mL). The other three petri dishes had ethanolic extract labeled as E1 (25mg/mL), E2 (50mg/mL), and E3 (100mg/mL). Perionyx posthuma worms were collected and washed with normal saline water. Then, three worms were placed in each petri dish with the leaf extract solution of FJ. From this study, we found that the leaf extract of FJ has anthelmintic activity. Higher concentration of the extract shows better effectiveness in killing worms for both the alcohol and water extracts of FJ. The extract from the plant leaf works better as a deworming agent than the standard medicine piperazine citrate.

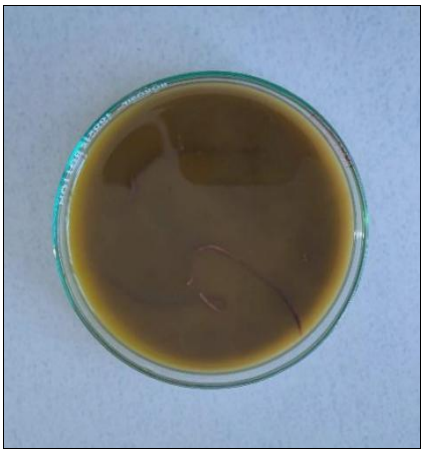
Result

The Result mark that *Flacourtia jangomas* shows potent anthelminthic activity, whereas *Perionyx posthuma* required a longer duration for mortality. The earth worm selected for anthelminthic study was highly sensitive to solvent extract ethanol leaf extract of *Flacourtia jangomas.* The anthelminthic activity results revealed dose dependent paralysis ranging from loss of motility to loss of response to external stimuli which eventually progressed to death at 25mg/mL, 50mg/mL, 100mg/mL concentrations. Paralysis time was observed respectively at 124, 63.33,53 min and death time 125, 65, 54 min in ethanolic extract. The paralysis time was observed respectively at 245.33, 128.33,71 min and death time at 246.33,130,74 min. The standard drug piperazine citrate shows paralysis within 26 min and time of death 25 min in the solvent extract. The observation of result show that the anthelminthic activity of ethanolic extract is more potent.

Aqueous leaf extract of *Flacourtia jangomas*

25mg/mL-A1

50mg/mL-A2



100mg/mL-A3

Alcoholic leaf extract of *Flacourtia jangomas*

25mg/mL-E1

50mg/mL-E2

100mg/mL-E3 Standard (25 mg/mL

Statistical Analysis

Comparison of time for paralysis using one-way ANOVA

Table 1: Descriptive statistics-paralysis time

Drug Extract	25mg/ml		50mg/ml		100mg/ml	
	Mean	SD	Mean	SD	Mean	SD
Aqueous extract of FJ	245.33	2.52	128.33	3.51	71.00	5.29
Alcoholic extract of FJ	81.67	5.03	49.67	7.77	32.67	1.53
Standard drug (Piperazine Citrate)	26.33	0.58	0	0	0	0

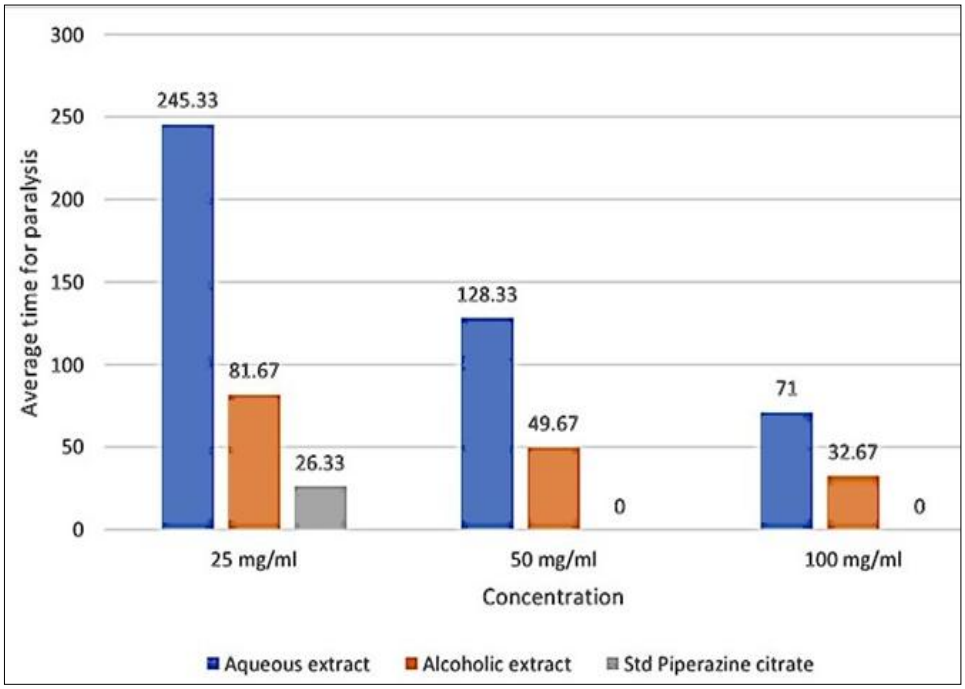


Fig 1: Graphical presentation of paralysis time in mins

Comparison of time for death using one-way ANOVA

Table 2: Descriptive statistics- death time

Drug Extract	25mg/ml		50mg/ml		100mg/ml	
	Mean	SD	Mean	SD	Mean	SD
Aqueous extract of FJ	246.33	2.52	130.00	4.00	74.00	3.61
Alcoholic extract of FJ	125.0	1.0	65.0	1.0	54.0	1.0
Standard drug (Piperazine Citrate)	24.67	0.58	0	0	0	0

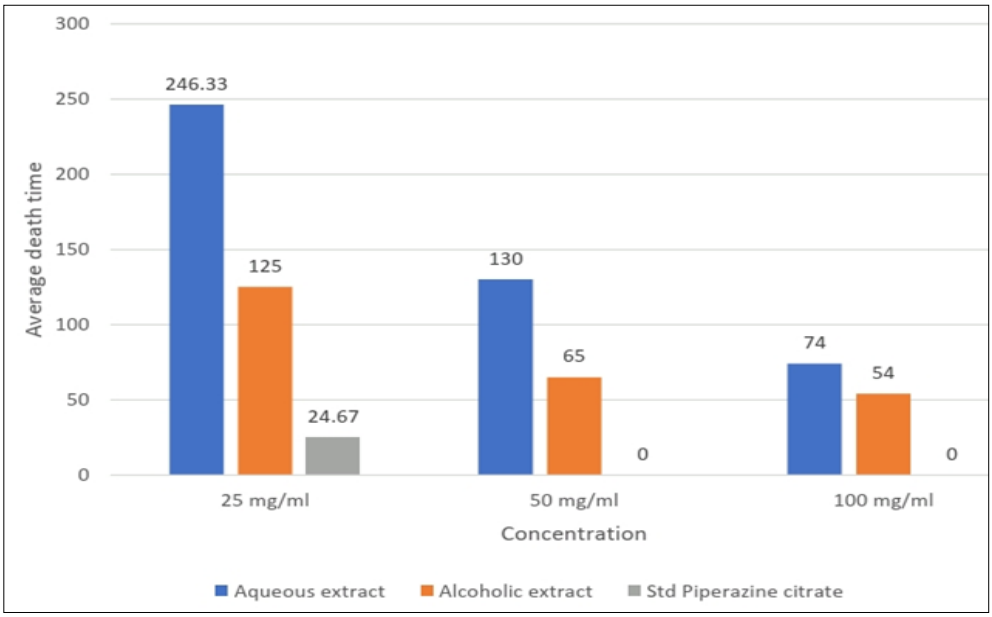


Fig 2: Graphical Representation of death time in mins

Comparison of total time (paralysis + death) using one-way ANOVA

Table 3: Descriptive statistics- total time (paralysis + death)

Drug Extract	25mg/ml		50mg/ml		100mg/ml	
	Mean	SD	Mean	SD	Mean	SD
Aqueous extract of FJ	491.67	5.03	258.33	7.51	145.00	8.89
Alcoholic extract of FJ	249.00	1.73	128.33	2.52	107.00	2.00
Standard drug (Piperazine Citrate)	51	1.00	0	0	0	0

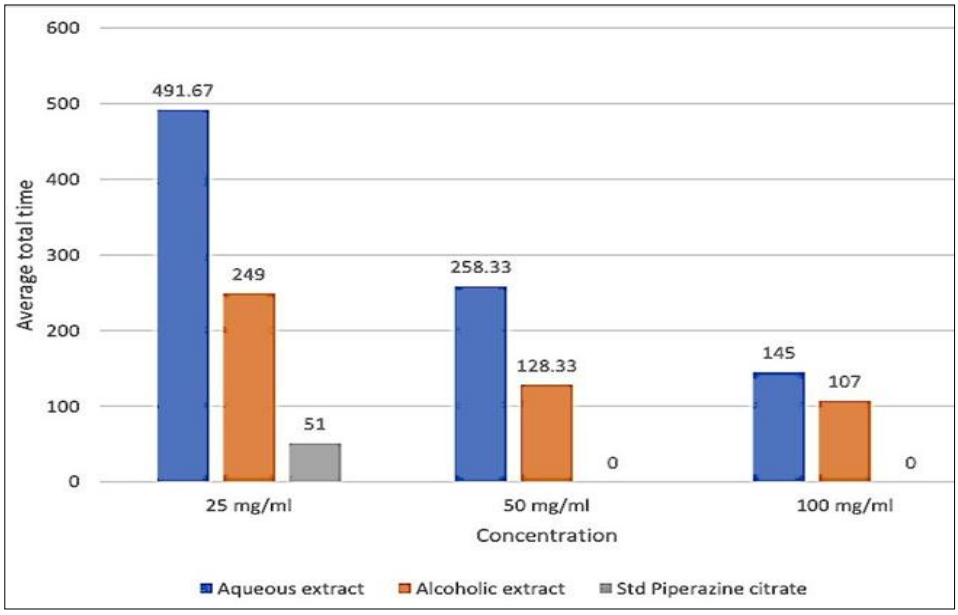


Fig 3: Graphical Representation of death time in min

Conclusion

The main purpose of the study was *In vitro* evaluation of anthelminthic activity of leaves extract of *Flacourtia jangomas* and compare the time of death and paralysis with that of the reference standard (piperazine citrate). The aqueous and alcoholic leaves extract of *Flacourtia jangomas* at various concentrations such as 25 mg/ml, 50 mg/ml, and 100 mg/ml was prepared and extract was poured into petri dishes containing *Perionyx posthuma* and time shows paralysis or death of *Perionyx posthuma*. The higher concentration shows less time taken for the paralysis or death of *Perionyx posthuma*. By this study we came to conclusion that 100 mg/ml concentration of alcoholic and aqueous extract of *Flacourtia jangomas* possess anthelmintic activity compare with the standard drug of piperazine citrate. This study is helpful for the future research on *Flacourtia jangomas.*

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