

In vitro Anti-Helminthic Study of *Ficus Dalhousiae* Leaf Extracts in *Pheretima posthuma*

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Received: 14th Aug, 18; Revised: 12th Sept, 18, Accepted: 15th Sep, 18; Available Online: 25th Sep, 2018

ABSTRACT

The aim of the present investigation was to evaluate the anti-helminthic activity of extracts of methanol, petroleum ether, n-hexane and water of *Ficus dalhousiae* Miq leaves in Earthworms. The plant material was collected from Tirupati was authenticated by Department of Botany, Osmania University, Hyderabad. Fresh plant leaves were used for the present study. The leaves were made free from dust and foreign material and dried and powdered. The powder was weighed (50 gm) and was extracted by successive solvent extraction process. Phytochemical screening was carried out for the detection of phytoconstituents. The parameters like paralysis time and time of death were determined by using the extracts of methanol, petroleum ether, n-hexane and aqueous solvent at the concentrations of 25mg/ml, 50mg/ml and 100mg/ml. The extracts of *Ficus dalhousiae* leaves exhibited significant antihelminthic activity at concentrations of 25mg/ml, 50mg/ml and 100mg/ml as compared with Albendazole as standard drug. The results from the study suggest that extracts of *Ficus dalhousiae* Miq leaves has shown significant anti-helminthic effect when compared to the standard drug, Albendazole.

Keywords: *Ficus dalhousiae*, Antihelminthic, Phytochemical, Extracts, Albendazole.

INTRODUCTION

Herbal Medicine or botanical medicine is the use of herbs for their medicinal value. Medicinal use of herbs in western cultures has its roots in the Hippocratic elemental healing system based on a quaternary elemental healing metaphor. Plants have been used for medicinal purpose since the ancient era¹. Helminthiasis, also known as worm infection, is a disease affecting humans and other animals in which a part of the body is infected with parasitic worms, known as helminths. Helminths are broadly classified into tapeworms, flukes and roundworms. Soil-transmitted heminthiasis and schistosomiasis are the most important helminthiasis and are among the neglected tropical diseases. This group of helminthiasis have been targeted under the joint action of the world's leading pharmaceutical companies and non-governmental organizations through a project launched in 2012 called the London Declaration on Neglected Tropical Diseases, which aims to control or eradicate certain neglected tropical diseases by 2020. The present study was initiated to evaluate anti-helminthic activity of methanolic extract of *Ficus dalhousiae* Miq leaves by using earthworms for their resemblance physiologically with the intestinal

roundworm parasite of human beings. *Ficus dalhousiae* Miq. (Moraceae) known as Kal Aal, Pei-Aal and Soma-valka to the locals is a traditional medicinal plant found in Tamil Nadu and Kerala states of India. *Ficus dalhousiae* is endemic to peninsular region² and is a rare species^{3,4}. Miquel described this species as *Urostigma dalhousiae* based on Wight's collection from India and later he named it as *Ficus dalhousiae*⁵. Literature indicates ethno medicinal use of this plant for hepatic and skin disorders. The present study was undertaken to investigate anti-helminthic activity of extract of *Ficus dalhousiae* Miq leaves.

MATERIAL AND METHODS

Material

The leaves of *Ficus dalhousiae* Miq (Moraceae) were collected from Tirupati, Andhra Pradesh and the plant material was authenticated by Department of Botany, Osmania University, Hyderabad. Earthworms were collected from Nizam College, Hyderabad. The standard drug Albendazole was procured from area medical store. All the solvents used for extraction were of analytical

grade and were purchased from Dhanalakshmi Scientific, Yousufguda, Hyderabad.

Preparation of Extract

Fresh leaves of the plant were dried and finely powdered and then passed from sieve #40. The dry powder was extracted with methanol, petroleum ether, n-hexane and aqueous solvent using process of soxhlet extraction⁶.

Phytochemical Tests

The preliminary phytochemical screening of the extracts was performed by the method described by Khandelwal.⁷

Evaluation of Anthelmintic Activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al.⁸. Adult earthworms (*Pheretima posthuma*) measuring around 3-6 cm in length were used to evaluate anthelmintic activity in vitro. Earthworms were washed with normal saline to remove faecal matter before starting of work. Test samples of the extracts of methanol, petroleum ether were prepared at the concentrations of 25, 50 and 100 mg/ml in distilled water. Albendazole (25 mg/ml) was used as the standard drug. Observations were made for the time taken for paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C followed with fading of their body colour.

RESULTS AND DISCUSSION

Results of Phytochemical Screening

Preliminary phytochemical screening of all the prepared extracts showed the presence of alkaloids, tannins, saponins, flavonoids and steroids.

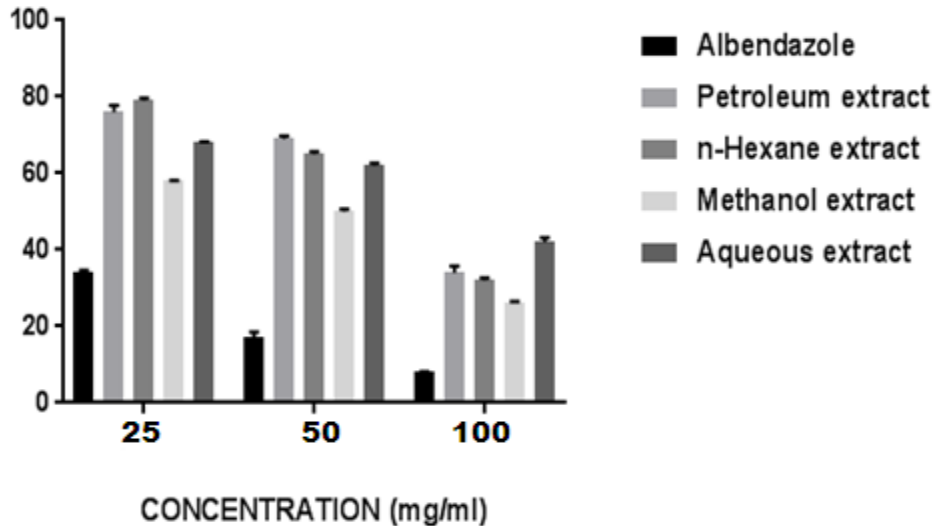
Results of Anthelmintic Activity

In the first experimental group (Albendazole) at 25mg/ml concentration the paralysis time was found to be (34±0.72) and the death time was (48±0.36). With increase in concentration of 50mg/ml there was a decrease in paralysis

time (17±1.55) and also death time (23±0.11). There was a drastic decrease in both paralysis and death time (8±0.22), and (14±0.45) respectively at the highest concentration of 100mg/ml. In the second experimental group (Petroleum extract) at 25mg/ml concentration the paralysis time was (76±1.83) and the death time was (80±0.64) which showed least pharmacological effect compared to the other groups. With increase in concentration of 50mg/ml the paralysis time was (69±0.81) and the death time was (78±0.40). There was a decrease in both paralysis and death time as (34±1.75) and (40±1.10) respectively at the highest concentration of 100mg/ml. In the third experimental group (Ethyl acetate extract) at 25mg/ml concentration the paralysis time was found to be (79±0.73) and the death time was (87±0.53). With increase in concentration of 50mg/ml the paralysis time was found to be (65±0.67) and the death time was (75±0.26). There was a drastic decrease in both paralysis and death time (32±0.74), and (37±0.34) respectively at the highest concentration of 100mg/ml. In the fourth experimental group (Methanol extract) at 25mg/ml concentration the paralysis time was found to be (40±0.15) and the death time was (65±0.92). At 50mg/ml concentration, the paralysis time and death time were found as (50±0.71) and (61±0.35) respectively. There was a maximum decrease in both paralysis and death time (26±0.55), and (33±0.77) respectively at the concentration of 100mg/ml which showed the maximum effect when compared to the standard drug, Albendazole. In the fifth experimental group (Aqueous extract) at 25mg/ml concentration the paralysis time was found to be (68±0.33) and the death time was (73±0.80). With increase in concentration of 50mg/ml the paralysis time was found to be (62±0.63) and the death time was (70±0.54). There was a drastic decrease in both paralysis and death time (42±1.17), and (55±0.11) respectively at the highest concentration of 100mg/ml. Table.1 & Graph.1

Table 1: Table depicting Anthelmintic activity of extracts at different concentrations

Group	Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
1.	Albendazole	25	34±0.72	48±0.36
		50	17±1.55	23±0.11
		100	8±0.22	14±0.45
2.	Petroleum extract	25	76±1.83	80±0.64
		50	69±0.81	78±0.40
		100	34±1.75	40±1.10
3.	Ethylacetate extract	25	79±0.73	87±0.53
		50	65±0.67	75±0.26
		100	32±0.74	37±0.34
4.	Methanol extract	25	40±0.15	65±0.92
		50	50±0.71	61±0.35
		100	26±0.55	33±0.77
5.	Aqueous extract	25	68±0.33	73±0.80
		50	62±0.63	70±0.54
		100	42±1.17	55±0.11



Graph 1: Graphical presentation of paralysis and death time in treated groups.

The values are in mean \pm SEM, from (n=6) in each group. The data is expressed as mean \pm SEM. The data was analyzed by one way ANNOVA followed by Dunnett's test. P-values <0.01 were considered significant.

DISCUSSION

Substantial advancements have been made during the previous decade and considerable number of synthetic drugs has been derived to sustain the damage caused by the helminth parasites. Unfortunately no effective medicine has been developed so far. The serious side effects of drugs and development of resistance has driven the severity of infection to a higher level. These factors paved way for herbal remedies as alternative antihelminthics⁹. Preliminary phytochemical screening of different extracts of leaves of *Ficus dalhousiae* revealed the presence of alkaloids, flavonoids, tannins and saponins. Tannins and polyphenolic compounds were shown to produce anthelmintic activities¹⁰. The triterpenoids may inhibit the food intake and cause paralysis and death of the organism¹¹⁻¹³. In vitro Anthelmintic activity was conducted with Petroleum ether, Ethyl acetate, Methanol and aqueous leaf extracts of *Ficus dalhousiae* leaves at the doses of 25mg/ml, 50mg/ml and 100mg/ml. The methanolic leaf extract of *Ficus dalhousiae* demonstrated death of worms in less time as compared to albendazole especially at higher concentration of 100 mg/ml. Methanolic extract produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli in a significant manner ($p < 0.01$). The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for Anthelmintic activity. The predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing

chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis¹⁴.

CONCLUSION

Ficus dalhousiae has shown significant antihelminthic activity. Further isolation and characterization of bioactive compounds from the plant is in progress.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Ahmed Baig, Member Governing Body, Anwarul Uloom Educational Association, New Mallepally, Hyderabad for providing necessary facilities for the present research work.

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