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EVALUATION OF *IN-VITRO* ANTHELMINTHIC ACTIVITY OF THE CRUDE HYDRO ALCOHOLIC LEAF EXTRACT OF *MUNTINGIA CALABURA LINN*

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ABSTRACT

The main aim of this study is to study the phytochemical screening and to evaluate *in-vitro* anthelminthic activity of the crude hydroalcoholic leaf extract of *Muntingiacalabura*Linn. using *Pheretimaposthuma*. The plant material was authenticated by botanist and extracted with hydroalcoholic mixture. Qualitative assay for the presence of plant phytoconstituents were carried out by following standard procedure. Hydroalcoholic extract from the leaves of *Muntingiacalabura* Linn. were investigated for their anthelmintic activity against *Pheretimaposthuma*. Six concentrations (1, 5, 10, 15, 20 and 25mg/mL) of extract were studied in activity, which involved the determination of time of paralysis of the worm. The extract shown positive results for the test of carbohydrates, proteins, sterols, flavonoids, alkaloids, saponins, glycosides and tannins. Albendazole (25 mg/mL) is used as a positive control and saline as negative control. The leaf extract exhibited a dose dependent anthelmintic activity and significant anthelmintic activity was found at highest concentrations of 20 and 25mg/mL. It was concluded from this study that the plant revealed significant anthelmintic activity at higherconcentrations and the possible mechanism may be due to the precipitation of proteins in the worms by tannins or impaired glucose uptake.

Key words: *in-vitro* anthelminthic activity, *Pheretimaposthuma*, *Muntingiacalabura* Linn. Hydroalcoholic extract, Tanins.

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INTRODUCTION

Helminthes infections are among the most common infections in humans, can affect most populations in endemic areas with major economic and social consequences. More than half of the population of the world suffers from various types of infection and majority of cattle's suffers from worm infections. *Muntingiacalabura* L. (family Elaeocarpaceae), the sole species in the genus *Muntingia*, is a flowering plant native to southern Mexico, the Caribbean, Central America, and western South America.

According to the Peruvian folklore, its leaves can either be boiled or steeped in water to provide relief from gastric ulcer or to reduce swelling of the prostate gland. The leaves, in particular, have been used to treat pain associated with gastric ulcers, headache, and cold or to attenuate the prostate gland swelling (1-3). Scientifically, the leaves of *Muntingiacalabura* L. have been reported to possess antitumour (4, 5), antinociceptive (6-8), anti-inflammatory and antipyretic (9), antibacterial (10), and antiproliferative and an antioxidant (11) activity of Muntingiacalabura L leaves. *Muntingiacalabura* L. has been traditionally used to treat many ailments. The present study aimed to determine the *in-vitro* anthelminthic activity of the crude aqueous leaf extract of *Muntingiacalabura* L. against *Pheretimaposthuma*.

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MATERIALS METHODS

Plant authentication and soxhlet extraction

The plant was identified and authenticated by Dr. B. Sandhya, M.Phil., Ph.D., Principal, SIMS College of Life Sciences, Mangaldas Nagar, and Guntur. The plant was collected, washed with water; shade dried,powdered, defatted with petroleum ether and extracted with mixture of 50% ethanol and 50% water using soxhlet apparatus for 18 h. The extract was filtered with whatmanfilter paper and solvent is evaporated to get the residue and it was used for preliminary phytochemical screening and *in-vitro* pharmacological evaluation.

Phytochemical screening

Qualitative assay for the presence of plant phytoconstituents such as glycosides, flavonoids, tannins and saponins were carried out on the powdered leaves following standard procedure as given in the table-1 (12, 13)

Table-1 Preliminary phytochemical tests for plant extract

Phytoconstituents	Test	Observation
Alkaloids (Hager's Test)	2mL extract + few drops of Hager's reagent	Yellow precipitate
Anthraquinones (Borntrager's Test)	3mL extract + 3mL Benzene + 5 ml NH_3(10%)	Pink, Violet or Red coloration in ammonical layer
Carbohydrates (Molisch's Test)	2mL extract + 10mL H_2O + 2 drops Ethanolic αnaphthol (20%) +2mL H_2SO_4 (conc.)	Reddish violet ring at the junction
Glycosides (Liebermann's Test)	2ml extract + 2mL $CHCl_3$ + 2mlCH_3COOH	Violet to Blue to Green coloration
Flavonoids	1ml extract + 1ml$Pb(OAc)_4$ (10%)	Yellow coloration
Proteins (Xanthoproteic Test)	1ml extract + 1ml H_2SO_4(conc.)	White precipitate
Saponins (Foam Test)	(a) 5ml extract + 5ml H_2O + heat	Froth appears
	(b) 5ml extract + Olive oil (few drops)	Emulsion forms
Steroids (Salkowski Test)	2ml extract + 2ml $CHCl_3$ + 2mlH_2SO_4 (conc.)	Reddish brown ring at the junction
Tannins (Braymer's Test)	2ml extract + 2ml H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate
Terpenoids	2ml extract + 2ml $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration
Phenol (Ferric chloride test)	2mlextract + 2ml of distilled water + 10 % $FeCl_3$ solution.	Bluish black colour

Standard drug used

Albendazole suspension (Micronized albendazole suspension in the concentration of 25 mg/mL).

Animal

The assay was performed on adult Indian earthworm, *Pheretimaposthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in-vitro* method.

Anthelmintic activity

Hydroalcoholic extract from the leaves of *Muntingiacalabura* Linn. (MCLE) were investigated for their anthelmintic activity againstIndian adult earthworms (*Pheretimaposthuma*). Various concentrations (1, 5, 10, 15, 20 and 25mg/mL) of leaf extract were tested in the bioassay, which involved determination of time of paralysis of the worms. Albendazole was included as standard reference and saline as control. The anthelmintic assay was carried as per the method of martin (14). *Pheretimaposthuma* collected from moist soil and washed with normal saline to remove all soil matters were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. In the first set of experiment, eight groups of six earthworms were released in to sufficient solutions of Albendazole, MCLE(25, 50, 100, 250, 500 and 1000µg/mL) in normal saline. Observations were made for the time taken to paralysis of individual worms. Time for paralysis was noted when there was no any sort of movement could be observed, except when the worms were shaken vigorously. The significant anthelmintic activity was observedat the concentration of 1000µg/mL. The second set of experiment was conducted with 1, 5, 10, 15, 20 and 25mg/mL in triplicates.

RESULTS AND DISCUSSION

Phytochemical screening of MCLE revealed the presence of carbohydrates, proteins, sterols, flavonoids, alkaloids, saponins, glycosides and tannins in the leaf extract,but the rests of test gave negative results as shown in table 2.

Table-2 Phytochemical analysis of extract of leaves of *M. calabura*

Phytoconstituents	Test result
Alkaloids (Hager's Test)	+
Anthraquinones (Borntrager's Test)	–
Carbohydrates (Molisch's Test)	+
Glycosides (Liebermann's Test)	+
Flavonoids	+
Proteins (Xanthoproteic Test)	+
phenolic compounds	
Saponins (Foam Test)	+
Steroids (Salkowski Test)	+
Tannins (Braymer's Test)	+
Terpenoids	–

(+) = Presence, (-) = Absence

MCLE shown anthelmintic activity from the concentration of 1000µg/mL but a significant anthelmintic activity was observed at the dose of MCLE-20 & 25 mg/mL in dose dependent manner as shown in Table-3 and figure-1. Albendazole is a vermicidal causes degenerative alteration in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. The loss of the cytoplasmic microtubules leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. Degenerative changes in the endoplasmic reticulum, the mitochondria of the germinal layer, and the subsequent release of lysosomes result in decreased production of adenosine triphosphate (ATP), which is the energy required for the survival of the helminthes. Due to diminished energy production, the parasite is immobilized and eventually dies. The extracts demonstrated paralysis as well as death of worms at a time comparable to albendazole especially at the concentration of 25mg/mL itself. Phytochemical screening of the crude extracts revealed the presence

of flavonoids and polyphenolic compound as one of the major chemical constituents. Polyphenolic compounds shown anthelmintic activity; chemically tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics e.g., niclosamide, oxyclozanide are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that tannins contained in the *Muntingiacalabura* extracts produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of theparasite and cause death (15).

Table-3 Anthelmintic effect of MCLE on *Pheretimaposthuma*

S.No	Groups	Quantity (mg/mL)	Time taken for paralysis (in min)
1.	Saline	0.9%Nacl	-
2.	Albendazole	25	31.67±6.506
3.	MCLE	1	384±8.000***
4.	MCLE	5	158±10.54***
5.	MCLE	10	86.33±6.506***
6.	MCLE	15	57.33±5.033**
7.	MCLE	20	45.33±5.508
8.	MCLE	25	38.33±3.512

Effect of MCLE onparalysis *Pheretimaposthuma*. Data are mean ± S.D (n=3). Statistical analysis was done by one-way ANOVA followed by Tukey's multiple comparison tests. $^{**}P$ <0.01, $^{***}P$ <0.001compared to albendazole. There is no significance difference between standard drug and MCLE-20 & 25 mg/mL.

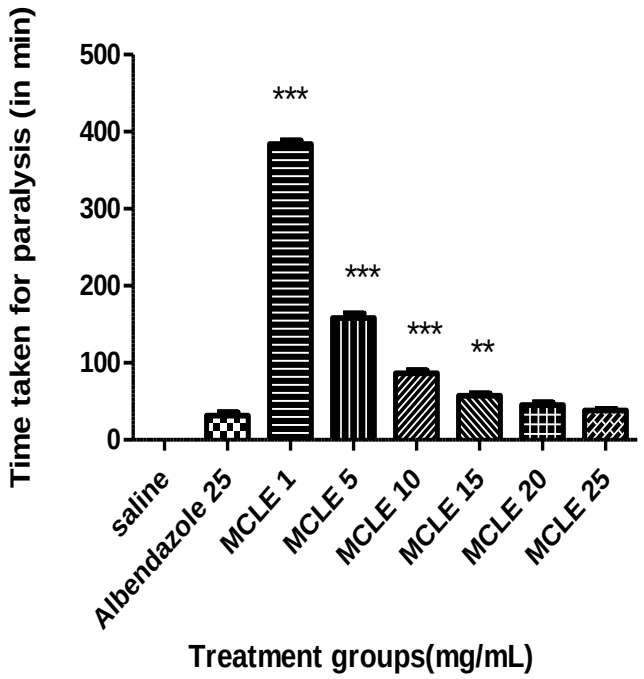

Figure-1 Plot of anthelmintic effect of theMCLE on *Pheretimaposthuma*

CONCLUSION

This study suggests that the hydroalcoholic leaf extracts of *Muntingiacalabura Linn* showed a dose dependent anthelmintic activity from 1mg/mL, but significant anthelmintic activity was found at the dose of 20 & 25 mg/mL. The experimental evidence obtained in laboratory model could provide a rational for the use of this plant as anthelmintic.The possible mechanism of action may be due to the precipitation of proteins in the worms by tannins or impairment of glucose uptake by the worms. Compound isolation characterization and possible mode of action to be studied to bring a novel anthelmintic drug.

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