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ANTHELMINTIC ACTIVITY OF CURCUMA CAESIA ROXB RHIZOME IN INDIAN ADULT EARTHWORM

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ABSTRACT
Curcuma caesia Roxb, popularly known as black termeric in India belongs to family Zingiberaceae. The objective of the present work was to evaluate the in-vitro anthelmintic potency of the ethyl acetate extract of *Curcuma caesia* rhizome using Indian earthworms (*Pheretima posthumad)*. The various concentrations (25, 50, and 75 mg/ml) of the ethyl acetate extract were tested in-vitro for anthelmintic potency by determination of time of paralysis and time of death of worm. Albendazole (15mg/ml) used as standard. The result of present study indicates that the *Curcuma caesia*. potentiate to paralyze earthworm and also caused its death after some time. The shortest time of paralysis was observed at higher dose (75 mg/ml) of ethyl acetate extract was found to 30.86 min. Thus, the present study demonstrate that the *Curcuma caesia* as an anthelmintic has been confirm as the ethyl acetate extract of leaves displayed activity against the earthworm used in study.

KEYWORDS: Curcuma caesia, Paralysis, death of earthworm.

INTRODUCTION
Helminthiasis or infections with parasitic worms are pathogenic for human beings. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelminthics are the drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues. Most of the existing anthelminthics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea.[1] Chemotherapy is the only treatment and effective tool to cure and control helminth infection, as effective vaccines against helminths have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites.[2] Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human[3] and could be of value in preventing the development of resistance.[4] *Curcuma caesia Roxb..Commonly* known as black termeric or Kali haldi and abundantly available in tropical and subtropical areas. Ancient history of India describes its diverse uses and also plays appreciable role in Ayurvedic or natural herbal medicines.[5] The plant has been reported to have analgesic, hypoglycemic, hepatoprotective. immunostimulant, anti inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, ntitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.[6,7] Traditionally, Curcuma caesia Roxb. claim as anthelmintic but scientifically it is not reveled still thus the present study was design to evaluate the in-vitro anthelmintic activity of ethyl acetate *extract* of Curcuma caesia Roxb.

MATERIAL AND METHOD
Plant: The fresh rhizome of Curcuma caesia Roxb. were collected in the month of December 2016 from its natural habitat at SFRI Forest region, Jabalpur M.P. India.

Experimental animals
All the experiments were carried out in Indian adult earthworms (*Pheretima posthumad*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity.[8] due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids*, of human beings.[9] Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in *vitro.*[10,11]

Material: Ethyl acetate extracts of Curcuma caesia Roxb, Albendazole (GSK. Ltd, Mumbai).

Preparation of Extracts of Curcuma caesia Roxb Rhizomes

The collected Rhizome of Curcuma caesia were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using soxhelet. The percent yield of ethyl acetate *extract* was 7.7% w/w. The extracts were concentrated by evaporation at room temperature and used for pharmacological studies.

Administration of Extract

The suspension of ethyl acetate *extract* of Curcuma caesia Roxb different concentrations (25- 75mg/ml) were prepared by using 0.2% v/v of Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration of\ Curcuma caesia Roxb. Five groups of approximately equal size worms consisting of four earthworms individually in each group were released into 10 ml of desired concentration of drug and extracts.

Administration of Albendazole

Albendazole (15mg/ml) was used prepared by using 0.2% v/v of Tween 80 as a suspending agent.

Experimental Design

Indian adult earthworms (*Pheretima posthumad*) collected from moist soil and washed with normal saline to remove all fecal matter were used for anthelmintic activity. Different concentration (25-75mg/ml) of Curcuma caesia *ethyl acetate extract* were prepared by using 0.2% v/v of Tween 80 as a suspending agent and final volume was made to 10 ml for respective concentration of Curcuma caesia. A albendazole (15mg/ml) was used as standard. The anthelmintic assay was carried out as per the method of (Ajaiyeoba et.al, 2001) with minor modification. The animals were divided into six group containing six earthworms each different concentration of extracts and standard drug solution were poured in different Petri dishesh. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when shaken vigorously nor when dipped in warm water (500c), followed with their body colors fading away).[13] All the results were expressed as Mean ±S.E.M. of four animals in each group. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by student's t test. At 95% confidence interval, p values < 0.001 were considered significant.[14]

Anthelmintic effect of Curcuma caesia Roxb in Indian adult earthworms (*Pheretima posthumad*)

For evaluation of anthelmintic activity Curcuma caesia Roxb Rhizome group I and II received normal saline and standard albendazole while group III, IV and V,VI received different concentration of ethyl acetate *extract* of Curcuma caesia respectively.

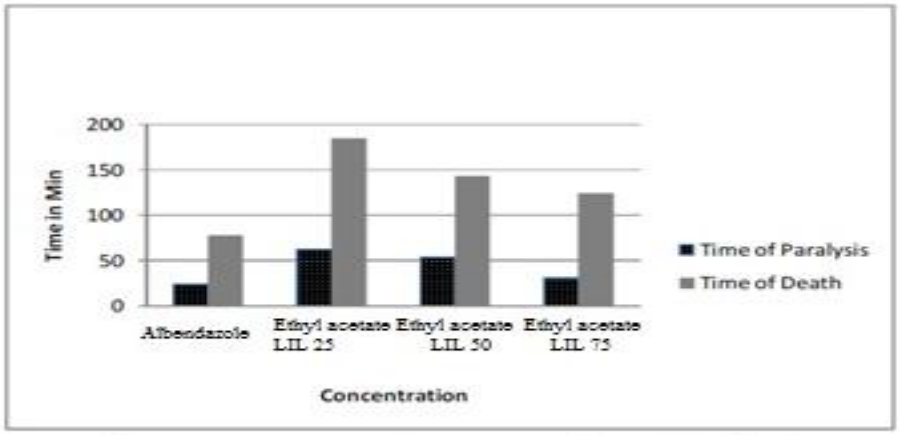
RESULT AND DISCUSSION

From the observation made in the Table 1, the ethyl acetate *extract* of Curcuma caesia was found to show anthelmintic activity when compared to standard drug. Curcuma caesia *extract* of rhizome of concentration 25, 50 and 75 mg/ml showed paralysis at 60.57, 51.13 and 28.86 min and death found to 170.20, 138.88 and 120.68 min respectively. Ethyl acetate *extract* of Curcuma caesia Roxb.showed paralysis of earthworm at 28.86 min at highest permissible dose (75mg/ml) which was comparable to standard albendazole (Table 1) From the above result, it is clear that ethyl acetate extract of Curcuma caesia have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug. It can be concluded that the active constituents responsible for anthelmintic activity present in the Ethyl acetate *extract* of. *Curcuma caesia* rhizome. Further study need to isolate and revealed the active compound contained in the crude extract of *Curcuma caesia* rhizome. and to establish mechanism(s) of action are required.

Table 1: Anthelmintic Potency of Curcuma caesia Roxb. rhizome

Drug/Treatment	Different Group	Concentration mg/ml	Time of paralysis (min) (Mean±S.E.M)	Time of paralysis (min) (Mean±S.E.M)
Normal Saline (Control)	I	-----	-----	-----
Albendazole	II	15	24.18±0.70	78.21±0.23
Ethyl acetate extract of Curcuma caesia Roxb.	III	250	60.57±0.63	170.20±1.41
	IV	500	51.13±0.63	138.88±0.68
	V	750	28.86±0.51	120.68±1.32

All values represent Mean ± SEM; n=6 in each group. Values are significantly different from reference standard (Albendazole) ***p<0.001. This activity was Concentration dependent. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms.



Ethyl acetate LIL- extract of Curcuma caesia Roxb.rhizome

Fig1- Anthelminthic activity of Curcuma caesia Roxb.

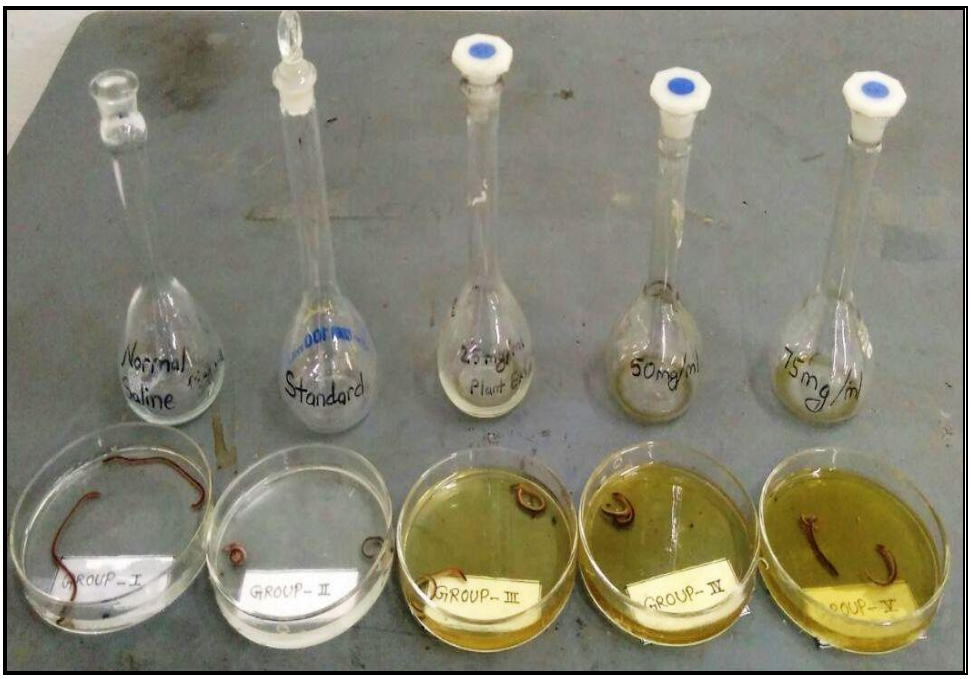


Fig-2 showing the five group earthworms with plant extract and standard

CONCLUSION

From the results it conclude that, Ethyl acetate *extract*s of Curcuma caesia Roxb.. demonstrate to possess dose dependant anthelmintic activity when compared to Albendazole. From results, Curcuma caesia. as an anthelmintic have been confirm as a it displayed activity against the worm used in present study. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms. The possible mechanism of the anthelmintic activity of Curcuma caesia. can not be explained on the basis of our present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis. Curcuma caesia may also have activated nicotinic cholinergic receptor in the worms resulting in either persistent depolarization or hyperpolarisation.[15]

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