IN VITRO ANTHELMINTIC ACTIVITY OF THE WHOLE PLANT OF *ENICOSTEMMA LITTORALE* BY USING VARIOUS EXTRACTS

S.Vidyadhar*, M.Saidulu, T.K.Gopal, D. Chamundeeswari, Umamaheswara rao, David Banji

*Department of Pharmacognosy, Nalanda College of Pharmacy, Osmania University, Nalgonda-508001, A.P., INDIA
Faculty of Pharmacy, Sri Ramachandra University, Chennai – 600 116, INDIA

ABSTRACT: Development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants has an alternative source of anthelmintics.In the current study, experiments were conducted to evaluate the possible anthelminitic effects of various extracts of the whole plant of *Enicostemma littorale.* Various concentrations (25, 50,100,200mg/ml) of all extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole was used as a reference standard and saline as a control group. Dose dependent activity was observed in all extracts *Enicostemma littorale.*
Key words: *Pheretima pothuma*, *Enicostemma littorale,* anthelminitic.

INTRODUCTION

Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries (Dhar *et al*., 1982) due to poor management practices. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths (Geert & Dorny, 1995; Coles, 1997) against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics (Satyavati *et al*., 1976; Lewis & Elvin-Lewis, 1977). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al*., 1956; Said, 1969; Akhtar *et al*., 2000; Iqbal et al., 2004).

In traditional system of medicine the practitioners use various indigenous plants for the treatment of anthelmintic. One such plant drug used by Siddha practitioners is *Enicostemma littorale* commonly called vellarugu is claimed by folklore for various ailments like rheumatism, skin diseases, and constipation.With this view, the *Enicostemma littorale* is studied for its anthelmintic property.

MATERIAL AND METHODS

The methodology adopted to evaluate the anthelmintic activity of various extracts of the whole plant of *Enicostemma littorale.* The plant specimens for the proposed study were collected from the Irulla Tribal women's welfare society**,** Thandarai, Chengalpattu, Tamilnadu and Authenticated by Prof.P.Jayaraman, Director, National Institute of Herbal Science(Reg.No of the certificate: PARC/2009/290)**.**

Plant material and preparations

Test drug: The whole plant of *Enicostemma littorale* was cut into small pieces dried in shade and powdered coarsely. The powdered *Enicostemma littorale* was extracted exhaustively with increasing polarity solvents (hexane, chloroform, ethyl acetate, ethanol, and water) for 72 hours followed by 48 hours and 24 hours. The solvents were pooled, distilled under vaccum and dried under vaccum dessicator.
Different concentrations (25, 50,100,200mg) of each of extract solution were prepared by diluting the stock solution, in propylene glycol, using normal saline.
Reference drug: Albendazole was prepared by dissolving them in normal saline at a concentration of 15mg/ml.
Experimental control treatment: A 10% propylene glycol in normal saline was used as experimental control treatment.
Normal control: Saline was prepared and used to treat the normal control group.

Worm collection and Authentication:

Pheretima pothuma (earth worm) nearly equal size (8cm) was collected from Manidharman Biotech Pvt Ltd, Porur,Chennai.

Anthelmintic activity

The anthelmintic activity was performed according to the method(Ghosh et al).On adult Indian earth worm Pheretima pothuma as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings.Pheretima pothuma was placed in petridish containing four different concentrations (25, 50,100,200mg) each of *Enicostemma* (hexane, chloroform, ethyl acetate, ethanol, and water extract)solutions. Each petridish was placed with 6 worms and observed for paralysis (or) death. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. In the same manner albendazole was included as reference compound. The Test results were compared with Reference compound Albendazole (15mg/ml) treated samples.

Table-1 In vitro anthelmintic activity of various extracts *Enicostemma littorale*

Group	Treatment of Extracts	Concentration (mg⁄ml)	Time taken for paralysis (min)	Time taken for death (min)
1.	Normal control	-	-	-
2.	Experimental control	-	-	-
3.	Albendazole (Reference)	15	38±0.3	56±0.3
4.	Hexane	25	50±0.2	64±0.5
		50	36±0.6	49±0.3
		100	27±0.4	33±0.8
		200	20±0.9	28±0.4
5.	Chloroform	25	45±0.1	59±0.6
		50	32±0.2	45±0.4
		100	25±0.1	30±0.1
		200	19±0.5	25±0.8
6.	Ethyl acetate	25	35±0.7	49±0.2
		50	27±0.6	39±0.4
		100	20±0.3	27±0.5
		200	15±0.9	22±0.6
7.	Ethanol	25	30±0.6	44±0.4
		50	26±0.4	38±0.2
		100	18±0.6	26±0.7
		200	13±0.5	20±0.4
8.	Water	25	40±0.2	54±0.2
		50	30±0.1	40±0.7
		100	22±0.5	29±0.2
		200	17±0.1	23±0.9

All Values represent Mean+ SD;n=6 in each group.
Comparisons made between standard versus treated groups.

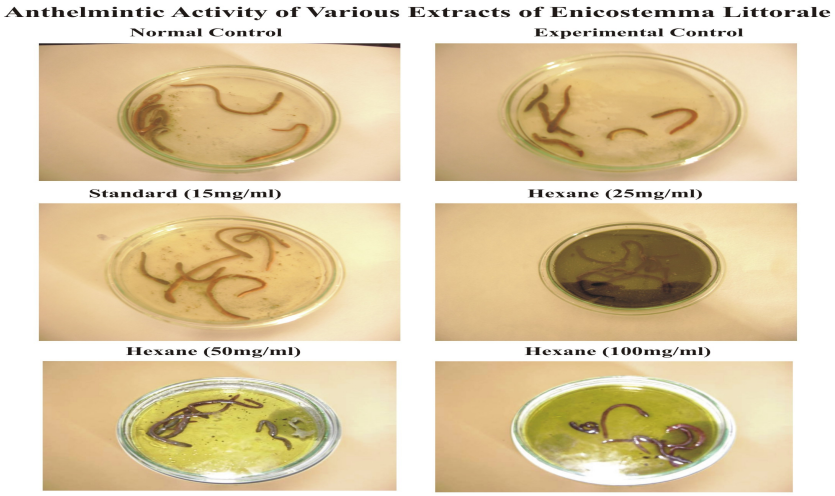
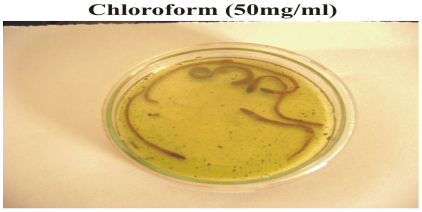


Fig A

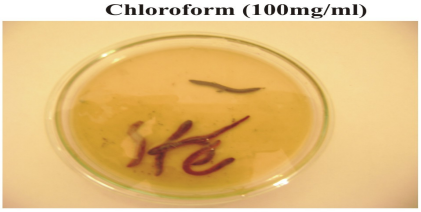
Hexane (200mg/ml)

Chloroform (25mg/ml)

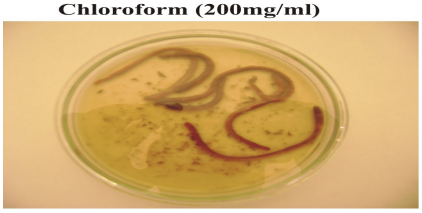
Chloroform (50mg/ml)



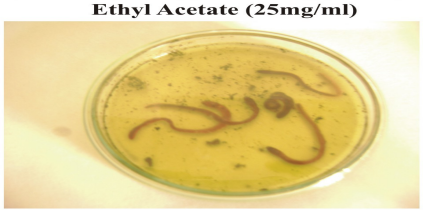
Chloroform (100mg/ml)



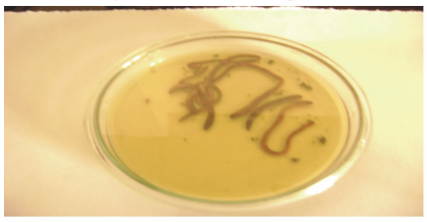
Chloroform (200mg/ml)



Ethyl Acetate (25mg/ml)



Ethyl Acetate (50mg/ml)

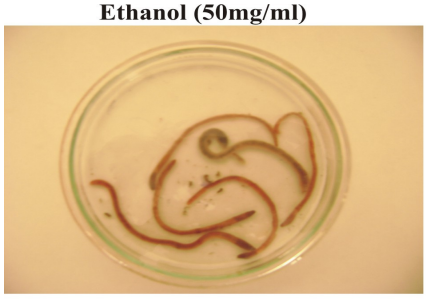


Ethyl Acetate (100mg/ml)

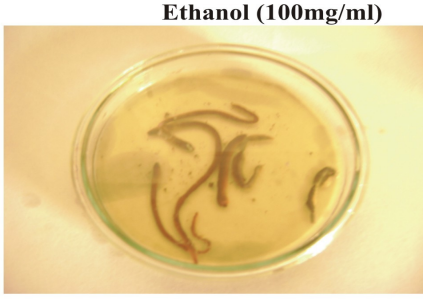
Ethyl Acetate (200mg/ml)

Ethanol (25mg/ml)

Ethanol (50mg/ml)



Ethanol (100mg/ml)



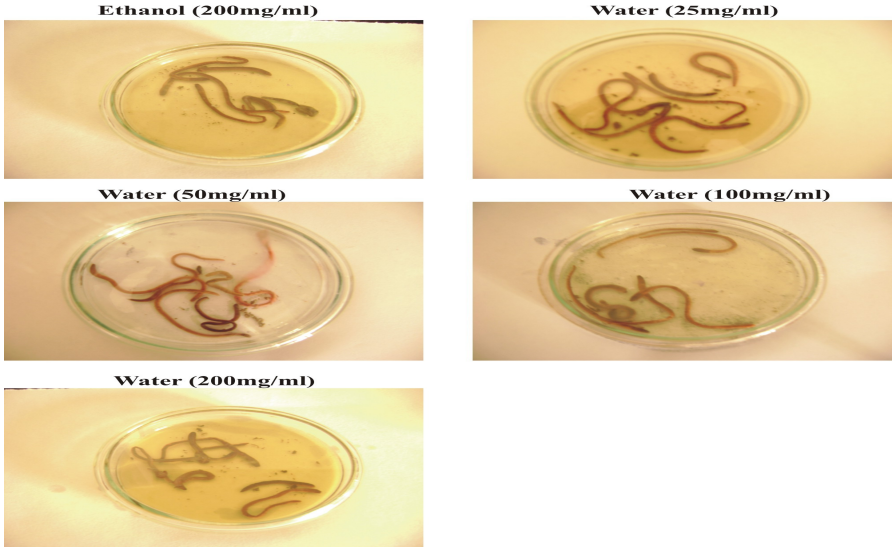


Figure-1: Effect of Extracts On The Paralysis (Or) Helminthiasis Of The Worm

RESULTS AND DISCUSSION

Preliminary phytochemical screening has shown the presence of alkaloids, tannins, flavonoids, triterpenes and reducing sugars.

The crude extracts samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations and the mean time values were calculated for each parameter. The crude extracts of ethanol showed the significant anthelmintic effect causing death of the worm at all the concentrations but the time of death was different in each case. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 25, 50, 100, and 200mg/ml. The ethanol extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of ethyl acetate,water,chloroform,hexane extracts. Similar observations were made in the anthelmintic activity as well.

The effect of extracts on the paralysis (or) helminthiasis of the worm, according to the results (table-1 and figure-1) may be indicated as ethanol>ethyl acetate >water >chloroform >hexane extracts. In particular the ethanol extract exhibited an increased paralytic as well as helminthiatic effect over albendazole at the given experimental concentrations (table1). This may be due to the increased level of extraction of tannins in ethanol followed by ethyacetate>water>chloroform>hexane extracts. The data presented in the table and observations made thereof, lead to the conclusion that the different degree of helminthiasis of the different extracts are due to the level of tannins present in compounds.

Tannins, the secondary metabolite, occur in several plants have been reported to show anthelmintic property by several investigators (Athnasiadou et al; 2001; Waller et al., 1997; Yesilada et al., 1993 ;). Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997) or, binds to the glycoprotein on the cuticle of parasite (Thompson and Geary, 1995), and cause death. Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post –translational modification prior to assembly into higher order complexes (or) ECMS (Page and Winter 2003). The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres.this results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannin of *Enicostemma littorale*, possibly by linking through hydrogen bonding, as proposed in this study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death. Hence further investigation and proper isolation of the active principles might help in the findings of new lead compounds, which will be effective against various parasitic infections.

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Conclusion

The wormicidal activity of various extracts of whole plant of *Enicostemma littorale* suggests that it is effective against parasitic infections of humans. Further, in future it is necessary to identify and isolate the possible active phytoconstituents responsible for the anthelmintic activity and study its pharmacological actions.

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