

COMPARATIVE EVALUATION OF ANTHELMINTIC ACTIVITY OF TWO PLANTS FROM THE FAMILY EUPHORBIACEAE

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ABSTRACT

The anthelmintic activities of aqueous, methanolic and acetone extract of *Acalypha indica* L. and *Euphorbia hirta* L. belong to the family Euphorbiaceae were evaluated separately on Indian adult earthworm *Pheretima posthuma* (Annelida). Extracts were prepared using soxhlet extraction and crude extract preparation methods with various concentrations (10, 25 and 50 mg/ml). Each extracts were tested in the bioassay, which involved determination of time of paralysis and time of the death of the worms. All extract were able to show anthelmintic activity at all concentration. The activities are well comparable with standard drug albendazole and piperazine citrate as positive control, distilled water (for aqueous extract) was used as negative control did not show any anthelmintic activity. The methanolic extract of both plant exhibited dose dependent anthelmintic activity showing maximum efficacy at 50 mg/ml concentrations. Methanolic and acetone extracts exhibit significant anthelmintic activity in present study. Methanolic extract of *Acalypha indica* L. have profound anthelmintic activity

Key words : *Acalypha indica* L. and *Euphorbia hirta*. *Pheretima posthuma*, Anthelmintic activity.

INTRODUCTION

People living in poverty in developing countries often suffer from helminthes infection. the most possible reason is poor sanitation, ignorance of hygiene, malnutrition, etc. This infection is common in man as well as in livestock which affect large population of the world which contribute under nourishment, anaemia, eosinophilia, pneumonia etc (Bundy, 1994). Helminth eggs usually lodge in the intestine, hatch, grow and multiply. They can sometimes infest other body sites. Anthelmintics or antihelmintics are the drugs that expelled worms from body either by killing (Vermicides) or stunning (vermifuge) them (Tripathi, 2004) but the emergence of resistance to anthelmintic drug

is now worldwide common fact which is the foremost problem in helminthiasis treatments. These drugs also produces toxicity to mankind therefore bioactive compounds derived from plants considered to be the best source against helminthes. Plants infusions, decoction, powder, juices are mainly preferred to against anthelmintics which have suggested to the proposal of screening medicinal plants for their anthelmintic activity. Due to good efficacy and cost effectiveness herbal medicine have gained much importance in recent years.

The herb *Euphorbia hirta* L. used for respiratory ailments, against worm infections in children, in jaundice (Kirtikar, et al.1933). The plant *Acalypha indica* L. also has cathartic,

anthelmintic, expectorant, emetic, antibacterial, anodyne and hypnotic properties, used to cure [Scabies](#). The leaves of this herb are used for treating vomiting, bronchitis, pneumonia and asthma. (Gangadevi, et al. 2008).

As per the literature people consume plants *Acalypha indica* L. and *Euphorbia hirta* L. belong to the family Euphorbiaceae to cure helminthic infections. Therefore this is an attempt to investigate these plants for its claimed anthelmintic activity.

MATERIALS AND METHODS

Plants resources:

The fresh plants *Acalypha indica* L. and *Euphorbia hirta* L. were collected in the months of Jan-Feb 2012 from the waste land and along roadside of GVISH campus, Amravati (M.S., India). The herbarium specimen were prepared and authenticated by the authority of department of botany, BAMU Aurangabad (M.S). A voucher specimen (0547 and 0548) was deposited in herbarium for future references. The whole plants were shade dried and coarsely powdered by a mechanical grinder. The materials were stored in an airtight container for further use.

Preparation of extracts:

Aqueous extract preparation:

The crude aqueous extracts (CAE) of *Acalypha indica* L. and *Euphorbia hirta* L. were prepared according to standard methods. 50 gram of the powdered plant material was mixed with 250ml of distilled water in 500 ml flask and boiled for 1.5 hrs and then allowed to cool. It was then filtered using whatman No.1 filter paper evaporated to dryness and stored at 4⁰c.

Methanol and Acetone extracts preparation:

Powdered plant materials were exhaustively extracted with methanol and acetone using soxhlet extraction method. The extracts were stored at 4⁰c until use. The crude extract (as much as needed) was dissolved in distilled water on the day of the experiment to prepare stock solution and different dilutions for the purpose of evaluating anthelmintic activity.

Drugs and chemicals:

Piperazine citrate and Albendazole (Ranbaxy Lab. limited) – as reference standards, Chemicals - methanol (70% v/v) and acetone were used during experimental protocol.

Test organism:

Mature and healthy earthworms (*Pheretima posthuma*) were used to evaluate the anthelmintic property of the extracted drugs. The earthworms were procured from Wadali forest nursery and washed with normal saline to remove all faecal matter and were used for the anthelmintic study. The earthworms of 3.5cm in length and 0.1-0.3cm in width were used for all the experimental protocol.

Anthelmintic assay:

Aqueous, methanolic and acetone extracts from the whole plant of *Acalypha indica* L. and *Euphorbia hirta* L. were investigated for their anthelmintic activity. Various concentrations (10, 25 and 50 mg/ml) of each extracts were tested in the bioassay, which involved determination of time of paralysis and time of the death of the worms. Albendazole and Piperazine citrate were included as standard reference and distilled water as control. The anthelmintic assay was carried as per the method of (Ajaiyeoba, et al. 2001) with minor modifications. In the first set of experiment, six groups of six earthworms were released into 25 ml of solutions of Albendazole, aqueous, methanolic and acetone extracts of whole plants of *Acalypha indica* L. and *Euphorbia hirta* L. The remaining groups were treated for different concentrations (25, 50 and 50mg/ml each in distilled water).

Piperazine citrate was used as reference standard while distilled water as control. All drug and extract solutions were freshly prepared before starting the experiment. Observations were made for the time taken to paralysis (P) and death (D) of individual worms. Time for paralysis was noted when the movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.

RESULTS AND DISCUSSION

The assay was advocated on adult Indian earthworms, *Pheretima posthuma* due to its easy availability and anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Sollmann, 1918; Vidyarthi, 1967; Thorn, et al. 1977; Suresh, et al. 2002) for the initial evaluation of anthelmintic compounds invitro.

Aqueous, methanol and ethanol extracts were used to evaluate anthelmintic activity, showed variable times at different concentrations. The mean \pm SEM values were calculated for each extracts. The result was given in table 1; reveal that different concentrations used for all extracts were compared in minimum concentrations with Piperazine citrate and Albendazole as reference drugs. The earthworm selected for anthelmintic activity was more sensitive to methanol extract of *Acalypha indica* (Graph 1, 2). At 10, 25 and

50mg/ml concentrations paralysis was observed respectively at 8, 6 and 5 min. Methanol extracts not only paralyzed the earthworm but also killed them earlier than other extracts. Piperazine citrate and Albendazole by increasing chloride ion conductance of worm muscle membrane responsible to muscle relaxation and finally paralysis (Rajeshwar et al, 2013; Martin, 1997).

From the observation, higher concentration of extract produced paralytic effect much earlier and the time taken for death shorter for both acetone and methanolic extracts. The methanolic extract of both plant exhibited dose dependent anthelmintic activity showing maximum efficacy at 50 mg/ml concentrations. The result shows more promising activity for acetone and methanolic extract as compared to aqueous extract of both plants as compared to standard (Table-1). Distilled water (for aqueous extract) was used as negative control did not show any anthelmintic activity.

Table 1: It shows an anthelmintic activity of various extracts of the *Acalypha indica* L. and *Euphorbia hirta* L.

Test sample	Concentrations (mg/ml)	<i>Acalypha indica</i>		<i>Euphorbia hirta</i>	
		P (in min.)	D (in min.)	P (in min.)	D (in min.)
Aqueous Extract	10	217 \pm 3.7	242.33 \pm 3.7	245.83 \pm 1.20	345.66 \pm 6.27
	25	147.33 \pm 4.5	164 \pm 6	223 \pm 1	268.16 \pm 4.35
	50	111 \pm 5.8	125.83 \pm 2.63	130.16 \pm 4.02	166.83 \pm 3.47
Methanol Extract	10	8.16 \pm 1.02	15.16 \pm 0.87	20 \pm 1.44	33 \pm 1.44
	25	7 \pm 1	13.33 \pm 1.40	17.16 \pm 0.87	29 \pm 2.22
	50	5.5 \pm 0.75	10 \pm 1	14.16 \pm 1.24	21.66 \pm 2.03
Acetone extract	10	14.16 \pm 1.02	28.16 \pm 1.20	55.16 \pm 2.36	63 \pm 1.66
	25	13.16 \pm 1.42	20.5 \pm 1.41	34.33 \pm 3.05	58.16 \pm 1.80
	50	9.16 \pm 1.02	15.83 \pm 1.42	30.83 \pm 1.53	42 \pm 1.16
Piperazine Citrate	10	21.5 \pm 1.41	29 \pm 1	23.66 \pm 1.05	29.66 \pm 1.55
Albendazole	10	36.33 \pm 1.72	53.5 \pm 1.25	37.66 \pm 2.07	55.33 \pm 2.74
Distilled water	----	----	-----	----	-----

Where, P= Time taken for paralysis of worms, D= Time taken for death of worms.

Values are expressed as mean \pm SEM, N=6. The results were analyzed by Analysis of variance (ANOVA).

The preliminary phytochemical analysis reveals that the plant contains flavonoid, phenols, saponin, steroids, alkaloids and tannins. Phenolic interference with glycoprotein of cell surface and this shows anthelmintic effect. Tannin along with this bind to free proteins in gastrointestinal tract as well as cuticle of parasite causes death of helminth (Patel, et al. 2010). Alkaloids may act on central nervous system and cause paralysis of worm (Barba, et al. 2010). Thus these phytochemicals promoting anthelmintic activity and its possible mechanism of action, therefore may these plants extract also produced similar effects.

CONCLUSION

The traditional used of *Acalypha indica* L. and *Euphorbia hirta* L. have been confirmed as its methanolic and acetone extracts exhibit significant anthelmintic activity in present study. This extract also proved to be efficient than standard drug. Therefore it can be concluded that methanolic and acetone extract of *Acalypha indica* L. have profound anthelmintic activity which justifies its folklore used in curing hemintic infections. Further efforts should be made to standardize the plant extract, isolate the possible phytoconstituent and formulate best alternative herbal to replace currently used synthetic drugs.

ACKNOWLEDGEMENT

The authors are grateful to Department of Botany, GVISH and Department of Govt. Pharmacy, Amravati for providing the facilities to carry out the entire experiment.

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DOI: <https://dx.doi.org/10.5281/zenodo.7206487>

Received: 5 April 2014;

Accepted: 21 May 2014;

Available online : 13 June 2014.