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Investigation to compare anthelmintic potentials of ethanol and aqueous extracts of Euphorbia hirta

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ABSTRACT
Objectives: The current investigation was performed for the comparative evaluation of ethanol and aqueous extract of Euphorbia hirta for its in vitro anthelmintic properties. **Methods**: The authenticated leaves of plant Euphorbia hirta were dried and powdered. The powdered drug was defatted with petroleum ether and a part of marc leftover was subjected to ethanol extraction using Soxhlet apparatus. Another part was subjected aqueous extraction using chloroform water. The ethanol and aqueous extracts of Euphorbia hirta was subjected to preliminary phytochemical investigation. Both the extracts were evaluated for in vitro anthelmintic properties using earthworms. **Results**: The ethanol and aqueous extracts were revealed for the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins and phenols. The paralysis time and death time of worms were significantly reduced by the administration of ethanol and aqueous extracts of Euphorbia hirta. **Conclusion**: The result of the present investigation confirms the anthelmintic property of Euphorbia hirta.

Key words: Anthelmintic activity, Euphorbia hirta, piperazine citrate, Pheretima posthuma, earthworms.

I. INTRODUCTION

The gastrointestinal (GI) tract's parasitic worms (helminths) are among the pathogens that have substantial global relevance. More than a billion individuals, especially in underdeveloped countries, are thought to be infected with helminths, which are transmitted through the soil. Helminth infection is a serious threat to cattle output and a significant threat to world food security[1,2]. A few handfuls of synthetic anthelmintic medications are essentially the only means of controlling helminths. The risks of parasites developing drug resistance (which is already common in some livestock production systems), the cost of drugs for small-scale farmers in developing countries, and for some helminths, the lack of efficacy of currently available drugs are the drawbacks of this reliance on chemotherapy. Therefore, there is an urgent need for additional and supplementary helminth control methods[3,4]. Humans and animals have historically used natural plant extracts as dewormers, but there hasn't been much scientific research to support this use or identify the active ingredients[5,6]. Plants' anthelmintic properties are typically attributed to secondary metabolites such proanthocyanidins, sometimes known as condensed tannins, alkaloids, terpenoids, or polyphenols[7,8]. Embellin, Procyanidins, prodelphinidins, and other proanthocyanidins are a diverse and common class of chemicals that are made up of polymers of catechin, epicatechin, and/or gallocatechin, with hetero-polymers being frequent. They have been extensively researched for their antioxidant and anti-inflammatory activities and can be found in both tropical and temperate plant material[9,10]. Since ancient period Ayurveda physicians Charka and Sushruta had mentioned the usefulness of several medicinal plants for the effective management diabetes with fewer side effects in Ayurveda, the traditional medicinal system of India. Herbal remedies for diabetes mellitus constituting of plant substances, either a single agent or in combination with other drugs, which are considerably safe and free from adverse reactions compared to synthetic agents[5]. Euphorbia hirta is an important medicinal plant that is used in a long period of time as herbal remedy to treat various diseases and ailments[11]. Different parts of the plant such as the stem, leaves, roots, seeds and barks are widely accepted worldwide in traditional setting and used as various form of remedies such as diuretic, astringent, anti-inflammatory, antibacterial, antihelmintic, etc. Modern researchers however showed that the various plant extracts has exhibited many potential as antibacterial, anti-fungal, anti-inflammatory, analgesic, antitumor and contraceptive ability[12]. These attributes are both related to an array of

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phytochemicals that are embedded within the plant parts for anthelmintic activities[13]. Hence, the present study is designed to test effect of ethanol extract of Euphorbia hirta for anthelmintic properties against earthworms.

II. 2.0 MATERIALS AND METHODS

Plant material

The areal part of Euphorbia hirta was collected from Foundation for Revitalization of Local Health Traditions (FRLTH) No.74/2, Jarakabande Kaval, Post Attur, Via Yelahanka, and Bangalore, Pin Code: 560106 Karnataka, INDIA. The plant was authenticated by Dr. Rama Rao, Scientist, Regional Ayurveda Research Institute for Metabolic Disorders.

Preparation of the ethanol extract

The leaves of the plant are dried under shade. The dried leaves are then powdered and 200gm of powdered drug was defatted with petroleum ether. A part defatted powdered drug was subjected to ethanol extraction in soxhlet apparatus for 48 hours and second part was subjected to aqueous extraction using chloroform water[14].

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanol (EEEER) and aqueous extract (AEER) of Euphorbia hirta was conducted as per procedure prescribed by Khandelwal[13].

Evaluation of in vitro anthelmintic activity of extract of Euphorbia hirta

Collection of Indian earthworms: Indian earthworm Pheretima posthuma (Annelida) were collected from the water logged areas of soil, the average size of earthworm being 6-8 cm. They were washed with tap water for the removal of the adhering dirt. The average sizes of the worms were 5-6 cm. The anthelmintic assay was performed on adult Indian earthworm Pheretima posthuma, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Pheretima posthuma worms are easily available and used as a suitable model for screening of anthelmintic drug [15,16]. The study consisting of 6 groups as follows

- **Group I-Normal**: Consisting of 6 worms treated with normal saline
- **Group II-Standard**: Consisting of 6 worms treated with 3 % of piperazine citrate
- **Group III-EEEH-low dose**: Consisting of 6 worms treated with 1 % of ethanol extract of Euphorbia hirta.
- **Group IV-EEEH-medium dose**: Consisting of 6 worms treated with 3 % of ethanol extract of Euphorbia hirta.
- **Group V-EEEH-high dose**: Consisting of 6 worms treated with 5 % of ethanol extract of Euphorbia hirta.
- **Group VI-AEEH-low dose**: Consisting of 6 worms treated with 1 % of aqueous extract of Euphorbia hirta.
- **Group VII-AEEH-medium dose**: Consisting of 6 worms treated with 3 % of aqueous extract of Euphorbia hirta.
- **Group VIII-AEEH-high dose**: Consisting of 6 worms treated with 5 % of aqueous extract of Euphorbia hirta.

The assay was performed on adult Indian earth- worm due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings. Various dilutions of standard drug (Piperazine citrate) and testwere prepared in normal saline (0.85%). Different concentrations of standard drug and test compounds in normal saline were poured into respective labeled Petri plates (50 ml in each plate) and 6 worms of equal size (or nearly equal) were introduced into each of the plates.

Parameters: Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was con- cluded when the worms lost their motility followed with fading away of their body colors. Death was also confirmed bydipping the wormsin slightlywarmwater. The mortality of parasite was assumed to have occurred when allsigns of movement had ceased.

Statistical Analysis

The data obtained from the present investigation were analyzed by ANOVA followed by post hoc Dunnet's t-test with the help of Graphpad prism5 software. All the values were shown as mean± standard error of mean (S.E.M.).

III. RESULTS

Preparation of extracts

The percentage yield of ethanol and aqueous extracts of Euphorbia hirta were found to be 9.09 and 8.22 respectively.

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Preliminary phytochemical study

The study revealed that, the ethanol and aqueous extracts of Euphorbia hirta consists of glycosides, alkaloids, flavonoids, tannins and phenolic compounds.

Table 1: Results of primary phytochemical analysis on EERH

Sl. No	Phytoconstituents present	Presence
1.	Carbohydrates	+
2.	Alkaloids	+
3.	Flavonoids	+++
4.	Glycosides	++
5.	Tannins	+
6.	Polyphenols	+++
7.	Proteins	++

Evaluation of in vitro anthelmintic activity of EEEH and AEEH

The results of anthelmintic activity of different concentrations of ethanol and aqueous extract of Euphorbia hirta are depicted in Table no. 5.3. The results revealed concentration dependent anthelmintic activity for both the extracts. The average paralysis time (in min) in different concentrations of Standard drug (Piperazine citrate) was found to be 21 mins while the average death time (in min) was found to be 31 mins. Ethanol extract of Euphorbia hirta in 1%, 3% and 5% was found to cause paralysis of worms in 73, 40 and 23 minutes respectively followed by death in 85, 47 and 28 minutes respectively. For aqueous extract of Euphorbia hirta in 1%, 3% and 5% was found to cause paralysis of worms in 68, 28 and 18 minutes respectively followed by death in 74,30 and 25 mins respectively and observed death in 74, 30 and 25 mins respectively . There were significant reduction in paralysis and death time were found in extracts treated group when compare to normal. The effect was comparable to that of piperazine citrate [Table 27 Fig 1-2].

Table 2: Effect on EEEH & AEEH on of paralysis time and death time of earthworms

Sl.No.	Group	Paralysis Time (mins)	Death Time (mins)
I.	**Normal**	127.1±3.61	164.02±9.05
I.	**Standard (3 % piperazine citrate)**	19.8***±1.51	29.5***±2.59
I.	**EEAH-Low dose (1%)**	71.7**±6.94	83.7**±5.38
V.	**EEAH-Medium dose (3%)**	39.7***±2.56	45.3***±2.47
V.	**EEAH-High dose (5%)**	21.3***±0.882	29.2***±2.82
I.	**AEAH-Low dose (1%)**	67.8**±3.76	73.2**±4.83
I.	**AEAH-Medium dose (3%)**	26.7***±2.09	30.0***±4.40
I.	**AEAH-High dose (5%)**	17.5***±1.48	23.5***±2.72

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Figure 1: Effect on EEEH & AEEH on time paralysis time of earthworms

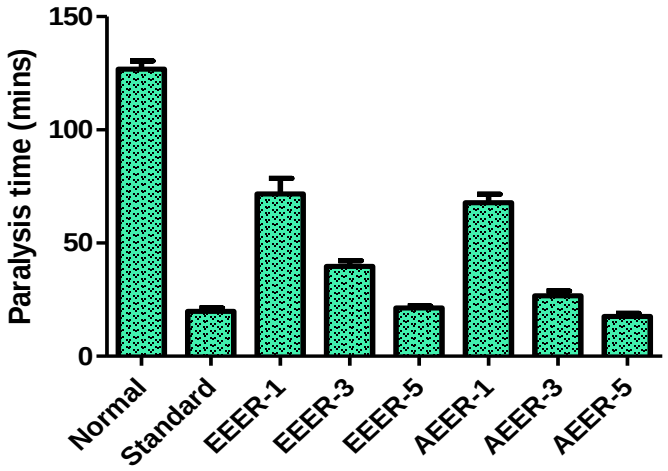
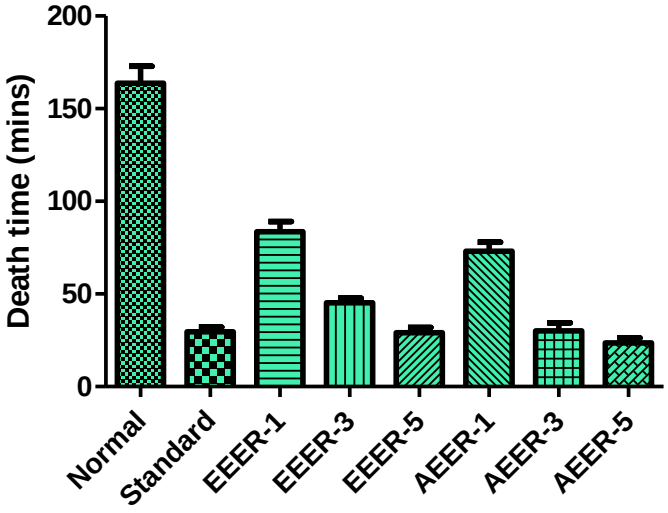


Figure 2: Effect on EEEH & AEEH on death time of earthworms



IV. DISCUSSION

Although there is availability of several pharmacological agents for the management of helminthiasis still there is no truly satisfactory drug for its effective management with least side effects. Hence identification and development of newer therapeutic agents remains highly desirable. In view of the toxicities effects and adverse reactions associated with the therapy using presently available anthelmintic drugs, searching for more potent and less toxic anthelmintic drug from plant origin is under pipeline throughout the world since herbal medicine play essential role in this segment due to their minimum side effects.

The present research study was conducted to evaluate ethanol and aqueous extract of Euphorbia hirta for its in vitro anthelmintic properties. The authenticated leaves of plant Euphorbia hirta were dried and powdered. The powdered drug was defatted with petroleum ether and a part of marc leftover was subjected to ethanol extraction using Soxhlet apparatus. Another part was subjected aqueous extraction using chloroform water. The ethanol and aqueous extracts of Euphorbia hirta was subjected to preliminary phytochemical investigation. Both the extracts were evaluated for in vitro anthelmintic properties using earthworms. The ethanol and aqueous extracts were revealed for the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins and phenols. The paralysis time and death time of worms were significantly reduced by the

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administration of ethanol and aqueous extracts of Euphorbia hirta.

V. CONCLUSION

The result of the present investigation confirms the anthelmintic property of ethanol and aqueous extracts of Euphorbia hirta. But the aqueous extract was more effective than ethanol extract. Further study should be conducted isolate and test specific constituents responsible for the anthelmintic activities and also to determine their mechanism of action.

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