

IN VITRO ANTHELMINTIC ACTIVITY OF *EUPATORIUM ADENOPHORUM* SPRENG LEAVES EXTRACT AGAINST *PHERETIMA POSTHUMA*

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Summary

Eupatorium adenophorum Spreng (Asteraceae) is found in the tribal area of Nagdhar and extensively used traditionally by the tribal people as wound healing, antiseptic, antimicrobial, and antifungal. The present study is an attempt to explore the anthelmintic activity of different extracts of leaves of plant *Eupatorium adenophorum* using petroleum ether, ethanol and chloroform as solvents and evaluated for their anthelmintic activities on adult Indian earthworms, *Pheretima posthuma*. All extracts were able to show anthelmintic activity at 2.5 mg/ml concentration. The activities are comparable with standard drugs, piperazine citrate and albendazole. All the doses of petroleum ether, ethanol and chloroform extracts of *Eupatorium adenophorum* showed better anthelmintic activity than the standard drug albendazole. The extracts of three solvent at concentration of 2.5 and 5.0 mg/ml showed lesser anthelmintic activity than the standard drug piperazine citrate. When the dose of the extract is increased, a gradual increase in anthelmintic activity was observed. The ethanolic extract showed better anthelmintic activity in comparison with petroleum ether and chloroform extracts. The data were verified as statically significant by using one way ANOVA at 5% level of significance ($p < 0.001$).

Key Words: *Eupatorium adenophorum*; Asteraceae; Anthelmintic; Piperazine citrate; Albendazole.

Introduction

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics.^[1]

Eupatorium adenophorum Spreng (Asteraceae) is a large genus of herbs, shrubs or under shrubs, distributed chiefly in tropical America, a few species occurring in Europe, Africa and Asia and India.^[2] Different parts of *Eupatorium adenophorum* Spreng are used in Ayurveda and other folk medicines for the treatment of cut and wounds. Leaves are used as an application to unhealthy ulcers. A decoction of the plant and the juice of the leaves are traditionally used as popular haemostatic remedy for various kinds of hemorrhage. Traditionally the leaves paste mix with mustard oil is useful for ulcer. Every part of the plant either alone or in combination has also been recommended for snake bite.^[3] The literature surevey revels that previously work has been done on different extracts of leaves for antimicrobial, anti- fungal^[4] and analgesic activity^[5] but there were no report on the anthelmintic activity of the leaves extracts of *Eupatorium adenophorum* Spreng. This prompted us to investigate the anthelmintic activity of *Eupatorium adenophorum* Spreng leaves extracts. *Eupatorium adenophorum* Spreng consist of various bioactive constituents like triterpenoids, flavanoides, sterols, saponins, triterpene alcohols and lactones.^[6]

Materials and Methods

Drugs and Chemicals

Albendazole and piperazine citrate (Sharon biomedicine pvt.ltd Dehradun), petroleum ether AR (60-80), chloroform, ethanol AR (CDH Pvt.ltd), normal saline water was used as control.

Plant Material

The Leaves of *E. adenophorum* Spreng (Asteraceae) were procured from the forest of Nagdhar Pokhari Chamoli (Uttarakhand) and identified Botanical Survey of India, Northern Regional centre, Dehradun (BSI) with the Accession number 1127802, 1127803. A voucher specimen has been preserved in the department for further verification.

Worm Collection and Authentication

The Indian earthworm *Pheritima posthuma* (Annelida) was collected from department of Zoology, DAV (PG) College Dehradun and authenticated.

Preparation of Plant Extracts

The leaves of *Eupatorium adenophorum* was extracted by using Soxhlet extraction apparatus. In the extraction procedure a total amount of 1000gm powdered leaves were extracted with each solvent. The solvents are petroleum ether, chloroform and ethanol. For each solvent, 50 cycles were run. Each extract was filtered and then it was concentrated by distilling of the solvent to

obtain the crude extract. Then it was dried by rotary evaporator. On successive solvent extraction of leaves of *Eupatorium adenophorum* with different solvents resulted in separation of constituents of different polarity range in different solvent extracts. The percentage yield of petroleum ether, chloroform and ethanol extracts of *Eupatorium adenophorum* was found to be 0.856 %, 3.856 % and 6.053 % w/w respectively.

Animals

Healthy adult Indian earthworm, *Pheretima posthuma* (Annelida) was used for evaluating the anthelmintic activity due to its anatomical and physiological resembles with the intestinal roundworm parasites of human beings. All earthworms were of approximately equal size.

Anthelmintic Activity

The anthelmintic activity was evaluated on adult Indian earthworm by the reported methods with slight modification.^[7] Eighteen groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were released in to 50 ml of respective formulation as follows: vehicle (1% gum acacia in normal saline), piperazine citrate (15 mg/ml), albendazole (10 mg/ml), chloroform (2.5, 5, 10, 25 and 50 mg/ml), ethanol (2.5, 5, 10, 25 and 50 mg/ml), petroleum ether (2.5, 5, 10, 25 and 50 mg/ml). The wide range of dose was taken to establish the relationship between dose and pharmacological activity and also to find out the minimum and maximum dose that can be better therapeutically effective in comparison to standard drug. Observations were made for the time taken to paralysis and / or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lose their motility followed with fading away of their body colour.

Statistical Analysis

The data on biological studies were reported as mean \pm Standard deviation (n=5). For determining the statistical significance, standard error mean and analysis of variance at 5% level significance was employed. P values < 0.001 were considered significant.^[8]

Result and Discussion

From the observation made each leaves extracts containing 2.5, 5, 10, 25 and 50 mg/ml, produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death as shown in Table 1. The petroleum-ether extracts of dose 2.5, 5, 10 and 25 mg/ml, produced paralysis within 35.567, 21.687, 16.39, 15.887 minutes respectively. Death was noted with 50 mg/ml concentration within 8.778 minutes. Ethanolic extract at concentration 2.5, 5, 10 and 25 mg/ml produced paralysis within 24.79, 16.38, 13.65, 11.76 minutes respectively. The death was noted with 50 mg/ml concentration within 7.42 minutes. Chloroform extract also showed dose dependent paralysis at concentration of 2.5, 5, 10 and 25 mg/ml, paralysis was noted at 30.683, 24.433, 20.65, 16.58 minutes respectively, while 50 mg/ml concentration produced death within 12.11 minutes. The standard drug Piperazine citrate of concentration 10 mg/ml and Albendazole of concentration 15 mg/ml shows paralysis at 18.56 minutes and 30.66 Minutes and death occurred after 60.78 minutes in case of Albendazole. The higher concentration of each extracts produced paralytic effect more quickly and time taken to death was shorter. By employing one- way ANOVA, all data were verified and found to be statistically significant at 5% level of significant (P < 0.001).

Table 1. Anthelmintic activity of leaves extracts of Eupatorium adenophorum Spreng

Groups	Treatments	Concentration used (mg/ml)	Time taken for paralysis (minutes) (X ± S.D.)	Time taken for death (minutes) (X ± S.D.)
1	Vehicle	-	-	-
2	Piperizine citrate	10	18.56 ± 0.31	-
3	Albendazole	15	30.66 ± 0.72	60.78 ± 0.79
4	Pet-ether extract	2.5	35.567 ± 0.79	-
5	Pet-ether extract	5	21.687 ± 1.78	32.19 ± 3.032
6	Pet-ether extract	10	16.39 ± 0.376	27.543 ± 1.069
7	Pet-ether extract	25	15.887 ± 0.376	21.432 ± 1.121
8	Pet-ether extract	50	8.778 ± 0.310	17.11 ± 1.67
9	Ethanol extract	2.5	24.79 ± 1.33	-
10	Ethanol extract	5	16.38 ± 0.508	23.48 ± 0.776
11	Ethanol extract	10	13.65 ± 1.751	23.32 ± 0.579
12	Ethanol extract	25	11.76 ± 0.562	18.32 ± 0.331
13	Ethanol extract	50	7.42 ± 0.589	14.5888 ± 0.2733
14	Chloroform extract	2.5	30.683 ± 0.811	-
15	Chloroform extract	5	24.433 ± 0.854	27.783 ± 0.331
16	Chloroform extract	10	20.65 ± 1.075	26.866 ± 0.388
17	Chloroform extract	25	16.58 ± 0.304	20.5 ± 1.165
18	Chloroform extract	50	12.11 ± 0.0813	15.3 ± 0.198
ANOVA Data			P-Value < 0.001	

Each values is represented as mean ± standard deviation (n=5)

Standard error means < 0.

Data are found to be significant by testing through one way ANOVA at 5% level of significance (P<0.001)

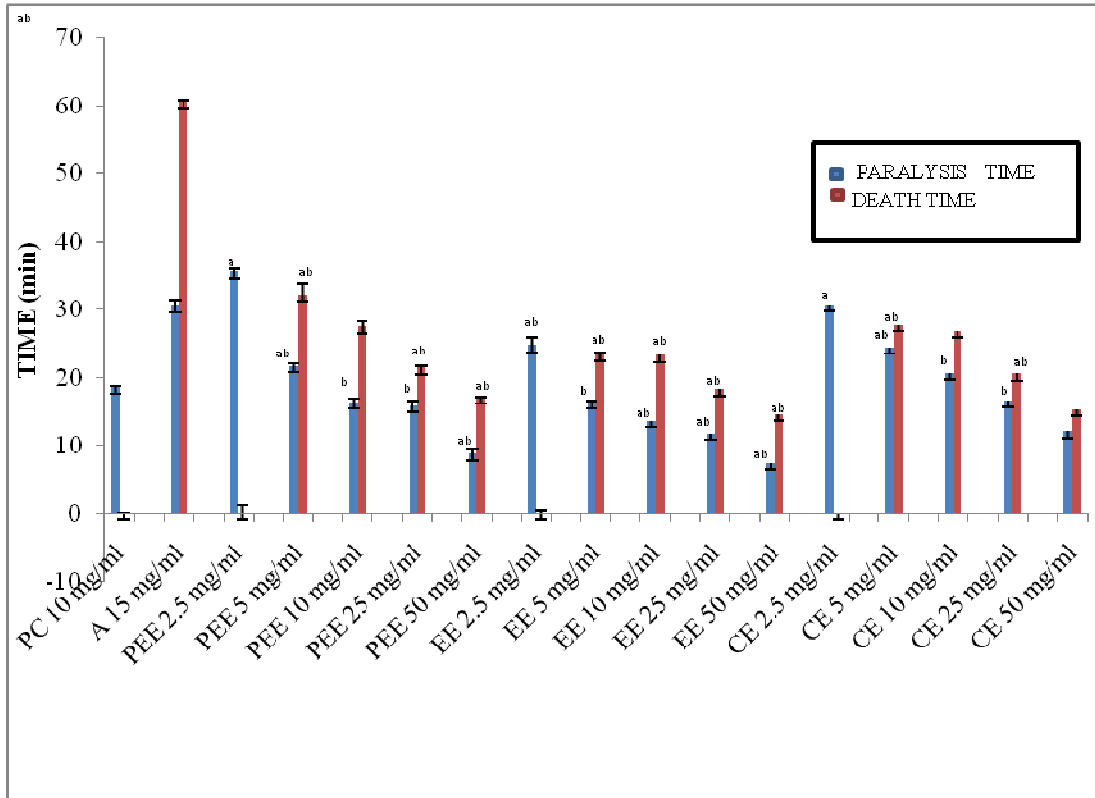


Fig-1: Graphical Presentation of In Vitro Anthelmintic Activities of *Eupatorium adenophorum* Spreng Leaves Extract against *Pheretima Posthuma*

p<0.001, a= significant compared with Piperazine citrate; b = significant compared with Albendazole

Each bar is represented as mean ± standard deviation (n = 5);

Group-1- Control (Normal saline water)

Group-2- Piperazine citrate (PC) 10 mg/ml (standard 1)

Group 3- Albendazole (A) 15 mg/ml

Group 4-8- Pet-ether extract (PEE) 2.5,5,10, 25, 50 mg/ml

Group 9-13- Ethanol extract (EE) 2.5,5,10, 25, 50 mg/ml

Group 14-18- Chloroform extract (CE) 2.5,5,10, 25, 50 mg/ml

From the above results, it was observed that the ethanolic extract was more potent than the other two extracts (petroleum-ether and chloroform) even though all the three extracts were endowed with anthelmintic property. The order of activity was ethanol extract greater than chloroform extracts greater than petroleum ether extract. The activity revealed concentration dependence nature of the different extracts. Potency of the extracts was found to be inversely proportional to the time taken for paralysis/death of the worms.

Conclusion

It could be concluded that the ethanolic extract showed most potent anthelmintic activity. The other two extracts e.g., petroleum ether and chloroform extracts, exhibited lesser anthelmintic activity than the ethanolic extract. The present study revealed that the anthelmintic activity increases with increasing polarity. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity and to establish the effectiveness and pharmacological rationale for the use of *Eupatorium adenophorum* Spreng as an anthelmintic drug.

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