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Research Article

Phytochemical Analysis and Potential Anthelmintic activity of *Chenopodium ambrosioides* Leaf Extract

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ABSTRACT

Helminthiasis illness is a common health risk for the population of all the ages throughout the world. The different major diseases caused by the infection are responsible for incremental mortality and morbidity statistics. From the older days in many civilizations the helminthes infection can be treated by different herbal medicines of dietary sources. This present study aimed to validate the traditional claim of anthelmintic activity of the herb Chenopodium ambrosioides. The petroleum ether extract of Chenopodium ambrosioides leaf (CALE) was used for the study and the presence of different phyto-constituents were observed. Indian earthworms Pheretima posthuman were incorporated in the different concentrations of Chenopodium ambrocioides leaf extract (5, 10 and 20 mg/ml). The paralysis time and the death time were noted and compared with the standard drug Albendazole. The phytochemical screening of the extracts gives positive indication of alkaloids, proteins, aminoacids, anhtraquinones, flavonoids, tannins, saponins, reducing sugar, steroids, triterpenoids and cardiac glycosides. The Chenopodium ambrocioides leaf extract in different concentrations showed significant anthelmintic activity. The potential of the effect was in the dose dependent manner. This study can be concluded as potential Anthelmintic activity of Chenopodium ambrosioides leaf Extract. Further studies are required to establish the fact clinically.

INTRODUCTION

A region of the body that has helminthiasis illness has been infected with nematodes like pinworms, roundworms, or tape worms. The worms typically live in the gastrointestinal system, but they can also enter the liver and various other body parts via body cavities. Despite the vast majority of worm-related infections are typically only found in tropical areas, several of them can appear in temperate climates and can affect travellers who have been there. They pose a serious risk to public health in emerging nations and raise the incidence

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of pneumonia, eosinophilia, anemia, and malnutrition. Three types of parasitic worms are distinguished: cestodes, also known as tapeworms or tape, nematodes, or roundworms, as well as trematodes, also known as flukes. Lymphatic filariasis, which causes elephantiasis, onchocerciasis, which results within river blindness, and schizophrenia are examples of parasitic illnesses that can have a serious morbidity impact . The majority of helminthes-related illnesses are chronic. More than any other group of parasites, they are likely to be the cause of morbidity and even poverty in people and other animals. Drugs known as anthelmintics work either locally to eliminate intestinal worms or systemically to eliminate adult helminthes or their developing forms that infiltrate organs and tissues. The majority of anthelminthic medications currently available cause side effects like diarrhea, headaches, nausea, vomiting, abdominal pain, and lack of appetite. The medication mebendazole is well taken. However, some patients have reported experiencing gastrointestinal adverse effects and dizziness. Additionally, extended use in cases of cysticerosis or dehydration results in fever, headache, alopecia, jaundice, and thrombocytopenia. It is crucial that we support research on conventional anthelmintic plants in order to create novel anthelmintic compounds that are readily available and have fewer negative side effects. This will help us eliminate the negative side effects of these synthesized anthelmintic drugs. Because of its morphological and cellular similarity to the intestinal roundworms parasites of humans and its accessibility, the anthelmintic activity of the mature Indian earthworms *Pheretima posthuma* (P. posthuma) was assessed.[1-4]

Plants such as *Coleus aromaticus* belongs to family Lamiaceae, often called Patta Ajwain, bulbils of *Dioscorea bulbifera L*., Leaf and Bark Extract of *Tamarindus Indica Linn* are also reported for having In vitro potential anthelmintic activity. [5,6]

Amaranthaceae's *Chenopodium ambrosioides L*. is a seasonal or annual plant with a strong fragrant smell. It is also commonly referred to as Mexican tea, Jesuit's tea, bluebush, Indian goosefoot or Spanish tea in English. It is widely used across west Africa, especially across Africa, Senegal, Ghana, and Congo. The plant is simple to grow and may thrive in a variety of soil types, including moderate (sandy), medium, heavy, acidic, neutral, and alkaline (pH 5.2 to 8.3). Although it cannot be grown in the shadow, it prefers damp soil [7,8]. It is typically found on cultivated land and arid wasteland. It is a domesticated and international species. *Chenopodium ambrosioides* is one of the most commonly utilized plants in traditional herbal remedies globally, according to the WHO . It is additionally widely utilized as an edible medicinal herb. (especially both leaves and seeds). Recent review studies have presented primary information on the pharmacological characteristics, phytochemicals, and conventional uses of *Chenopodium ambrosioides*.[9,10]

In numerous traditional and folklore medical systems, this herb has been used as a treatment for a variety of illnesses, including skin infections, ocular problems, poison stings, stomachaches, neurological disorders, repair wounds, anti-inflammatory, anti-tumor, and rheumatism.[11]

Till now no studies have been performed to evaluate the anti-anthelmintic activity of *Chenopodium ambrosioides*. So the present study has been designed to investigate possible anti-anthelmintic activity of *Chenopodium ambrosioides* using earthworms.

MATERIAL AND METHODS

Collection of Plant Material

During the months of March and April, 2023 *Chenopodium ambrosioides* leaves were collected from the local market of Singtam, East Sikkim, Sikkim, India. A total of 250 grams of mature and

young leaves were extracted, completely washed with deionized water, and shade dried. The leaves were separated from the stem once they have dried completely, ground into a fine powder in a grinder, and then stored in airtight containers for future research.[12]

Extraction of Plant Material

Approximately 25 g of powdered crude drug of *Chenopodium ambrosioides* leaves were extracted by soxhlet apparatus, with petroleum ether as solvent for 16 hours. The solvent was recovered after extraction and the extracts were concentrated by rotary evaporator at temperature between 40-45°C .[12]

Qualitative Analysis of theExtract

The aqueous extract of *Chenopodium ambrosioides* leaves were subjected to qualitative analysis for the following organic plant constituents: alkaloids, proteins amino acids, anthraquinones, flavonoids, saponins, tannins, steroids, triterpenoids and cardiac glycosides.

Test for alkaloids

- **Mayer's test**

A test tube was filled with 2ml of the extract. 0.2ml of diluted hydrochloric acid and 0.1ml of Mayer's reagent was added. Formation of yellowish buff coloured precipitate indicates the presence of alkaloids.

Test for Proteins and Amino Acids

- **Millon's test**

The extract was treated with 2ml of Millon's reagent. The formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acids.

Test for Anthraquinones

- **Modified Borntrager's test**

The extract was treated with ferric chloride solution and heated on a boiling water bath for 5 min. After cooling, the mixture was shaken with an equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. The formation of rose pink or cherry red color in the ammoniacal layer indicates the presence anthraquinones.

Test for flavonoids

5ml of the extract was hydrolyzed with 10% v/v sulphuric acid and cooled. Then it was extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted ammonia, 1ml of diluted sodium bicarbonate and 1ml of 0.1N sodium hydroxide were added to the first, second and third test tubes respectively. Formation of yellow colour in each test tube indicates the presence of flavonoids.

Test for reducing sugars

- **Felhing's test**

5ml of extract solution was mixed with 5ml of Fehling's solution (equal mixture of Fehling's solution A&B) & boiled. The presence of reducing sugars is indicated by the formation of brick red precipitate.

Test for saponins

- **Foam formation test**

1ml solution of the extract was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. The development of stable foam indicates the presence of saponins.

1ml of the extract was treated with 1% aqueous lead acetate solution. Appearance of white precipitate indicates the presence of saponins.

Test for tannins

5ml of extract solution was allowed to react with 1ml of 5% ferric chloride solution. Formation of greenish black indicates presence of tannins.

Test for steroids, Triterpenoids and Cardiac glycosides

- **Liebermann-Burchard test**

10mg extract was dissolved in 1ml of chloroform, 1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid from the sides of the test tube. The formation of a reddish violet colour at the junction denotes the presence of cardiac glycosides, triterpenoids, and steroids.

Selection of Indian Earthworms for Experiment

Although Indian earthworms *Pheretima posthuman* (Annelida) resemble human intestinal roundworm parasites anatomically and physiologically, they were selected to carry out the anthelmintic activity. The worms were gathered from the water logged areas of soil and cleaned with regular saline for removal of the adhering. To conduct the experiment, worms that were between 0.2 and 0.3 cm in width and between 3-5 cm in length were used.[13]

DRUGS AND CHEMICALS

Albendazole purchased from local medical shop was used as standard drug. As test drugs for the activity, *Chenopodium ambrosioides* petroleum ether extract(PEE) was utilised. The experimental protocol uses grade chemicals and solvents.

Experimental Procedure

Some adjustments were made to the Ajayieoba et al procedure for the anthelmintic assay before it was completed. For the in-vitro anthelmintic bioassay of PEE, Indian earthworms *Pheretima posthuman* (Annelida) measuring average size of 6-8cm were utilised. Briefly, six the same-sized earthworms were placed in 20 ml formulations containing three different concentrations of crude petroleum ether extract (5, 10 and 20 mg/ml in double-distilled water). The 'time for paralysis' was noted when no movement of any sort could be seen other than when the worms were vigorously shaken in both the test solution and standard drug solution. The 'time for death' of the worms was determined after it was discovered that they did not move when shaken vigorously or when immersed in warm water at 50°. The paralysing and death times of *Pheretima posthuma* and worms were determined to be 120 minutes at the most. Albendazole (5,10 & 20 mg/ml) was used as reference standard with distilled water as the vehicle control.[14]

Statistical Analysis

All experiments were repeated thrice. By using an ANOVA followed by a Tukey Kramer multiple comparision test, the mean and SEM were statistically analysed, $P<0.05$ being considered as significant.[15]

RESULTS

Collection of plant material: Plant material or leaves of *Chenopodium ambrosioides* has been collected from the local market of singtam.

Drying: The leaves of *Chenopodium ambrosioides* has been dried in shade and powdered by grinder.

Extraction: The extraction of *Chenopodium ambrosioides* was extracted using soxhlet apparatus with petroleum ether as a solvent. The percentage yield was found to be 4.49 % w/w.

Solvent recovery: Solvent recovery was performed using rotatory flash evaporator.

Phytochemical Estimation:

The phytochemical estimation of *Chenopodium ambrosioides* leaf extract (CALE) was performed and found the presence of alkaloids, proteins, amino acids, flavonoids, reducing sugar, tannins.

Table: 1 Phytochemical tests for the presence of active constituents in Chenopodium ambrosioides leaf extract

Test for active constituents	GTE
1. Alkaloids	+
2. Proteins	+
3. Amino acids	+
4. Anthraquinones	_
5. Flavonoids	+
7. Reducing sugar	+
8. Saponins	_
9. Tannins	+
10. Steroids	+
11. Triterpenoids	_
12. Cardiac glycosides	_

+ : Positive test ; _ : Negative test

Anthelmintic Activity:

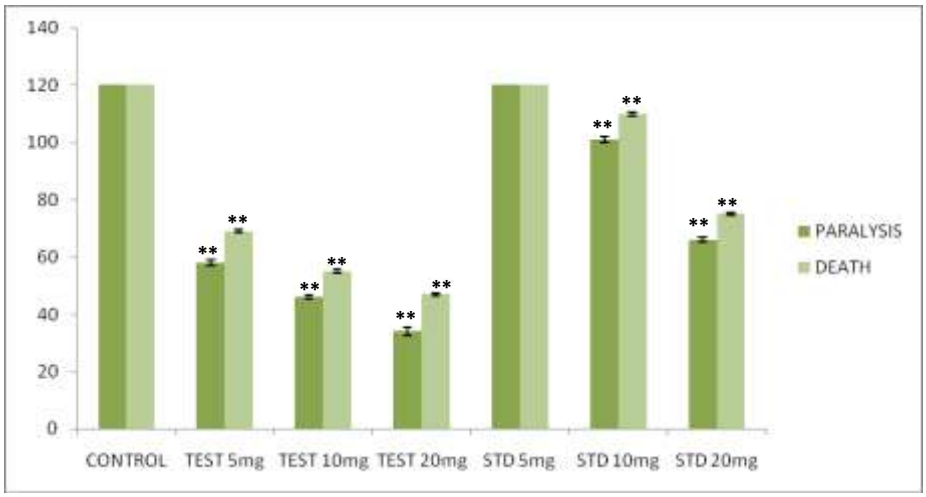
The petroleum ether extract of *Chenopodium ambrosioides* leaf extract (DALE) in different doses of 5mg/ml, 10mg/ml and 20 mg/ml showed significant ($p<=0.001$) decrease in time for paralysis and death compared with the control group. The response

observed in the extract witnessed in a dose dependent manner. Eventually, this paralysis led to death. For the extracts, the Mean S.D. values (statistical analysis) had been obtained. The results of the anthelmintic activity on the earthworm *Phertima prosthuma*, which are presented in Table I, show that different concentrations of petroleum ether extracts resulted in the paralysis and death of earthworms when compared to control group. The extract has taken the time of (36.33 ±1.45) min for paralysis and time of death was (48.00 ± 0.58) min, and the standard drug has taken the time of (66.33 ± 0.88) min for paralysis and time of death was (75.00 ± 0.57) min. The results showed that petroleum ether extract in the concentration of 20 mg/ml has taken less time to cause paralysis, and little more time to cause death of earthworms.

Table No: 3 Drug treatment & time of death

Group	Drug treatment	Conc. Mg/ml	Time for Paralaysis (min)	Time of death (min)
I	Albendazole	20mg/ml	66.33 ± 0.88***	75.00 ± 0.57***
II	Albendazole	10mg/ml	100.66 ± 0.88***	111.00 ± 0.57***
III	Albendazole	5mg/ml	120.00 ± 0.00	120.00 ± 0.00
IV	Plant extract	20mg/ml	36.33 ± 1.45 ***	48.00 ± 0.57***
V	Plant extract	10mg/ml	45.33 ± 0.66***	55.66 ± 0.66***
VI	Plant extract	5mg/ml	56.66 ± 0.88***	69.00 ± 0.57***
VII	Saline water	No mortility	120.00 ± 0.00	120.00 ± 0.00

***P≤ 0.001when compared with the control group, Test:*Chenopodium ambrosioides,* Std:Albendazole



***P≤ 0.001when compared with the control group, Test:*Chenopodium ambrosioides,* Std:Albendazole

Figure No: 1 Effect of Chenopodium ambrosioides on death and paralysis time

Figure No: 2 Earthworm Treated with saline water

Figure No: 3 Earthworm treated with drug

Figure No: 4 Death of Earthworm by treating with standard drug

Figure No: 5 Death of Earthworm by treating with test drug

DISCUSSION

Some of the traditionally used herbs have scientifically proved a potent anthelmintic activity by using suitable experimental models. Alcoholic and aqueous extracts from the roots of the plant *Baliospermum montamum Muell*. had shown significant anthelmintic activity at concentration of 100mg /ml with reference to piperazine citrate (10mg/ml) as a standard drug by using adult earthworms *P. posthuma* and *Ascardia galli* as experimental model (Mali et al., 2008). Polyherbal formulation containing aqueous and ethanolic extracts of *Plumbago zaylanica* (leaves), *Hyocymusniger* (roots), and *Abultion indicum* (leaves) has shown anthelmintic activity using adult earthworms *P. posthumaas* experimental model (Chaturvedi et al.,2009).[16] The present study reveals that leaves of *Chenopodium ambrocioides* showed mark and potent anthelmintic activity. In previously studied on *Chenopodium ambrocioides* had shown that the extraction process with petroleum ether gives promising result and maximum extracts from the leaves. [16] In our present study also the percentage yield was found to be 4.49% w/w. The phytochemical screening of the extracts gives positive indication of alkaloids, proteins, aminoacids, flavonoids, tannins, reducing sugar, steroids, which is similar as found in the earlier literatures. [16] The *Chenopodium ambrocioides* leaf extract (CALE) with the dose range of 5, 10 & 20 ml were used for the evaluation of anthelmintic activity. The results of the present study showed potential anthelmintic activity by significant reduction in the duration of paralysis and death time. The experimental results of extract treated groups were comparable with the standard drug albendazole which also use in same concentration in distilled water. The *Chenopodium ambrocioides* leaf extract witnessed the potential anthelmintic activity may be due to the presence of potential phytoconstituents like alkaloids, proteins, amino acids, flavonoids, tannins, steroids etc. The result of our present research is very promising and it can provide an alternate therapy as an anthelmintic agent. The leaves of this plant can be further investigated and established as anthelmintic herbal medications because they are affordable and easily accessible in local markets.

CONCLUSION

According to the a fore-mentioned finding, petroleum ether extract of *Chenopodium ambrosioides* leaves exhibits powerful anthelmintic action when compared to commonly

prescribed medication as it is comparable to the normal drug. Further detailed investigation using an in vivo model can be beneficial to determine the efficacy and establish the pharmacological basis for the therapeutic application of leaves as an anthelmintic medication. Further, the probable mechanism of action also can be evaluated by separating the active ingredient from the extracts.

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