DOI: https://doi.org/10.31782/IJCRR.2022.141904



Evaluation of *In Vitro* Anthelmintic Activity and Phytochemical Analysis of Ethanolic Extract of *Cardiospermum halicacabum* Leaves

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ABSTRACT

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Introduction: The infection caused by helminths is called as Helminthiasis. It is otherwise known as helminthosis, helminthiases, helminth, infection and worm infection. They often live in the gastrointestinal tract of their host, but they may also burrow into other organ, where they induce physiological damage. The different categories of worms consist of Roundworms, Pinworms, Threadworms, Whipworm Hook worm, Flat worm and Tapeworm.

Objective: The present study was carried out to find the anthelmintic activity of Cardiospermum halicabum.

Methods: The anthelmintic activity of *Cardiospermum halicacabum* was analyzed by in vitro method by using the Indian earthworms. The activity was performed and the paralysis time and death time were noted down. The anthelmintic activity of the extract was compared with that of the standard drug Albendazole.

Results: The ethanolic extract at the concentrations of 100 mg/ml, 200 mg/ml, 300mg/ml showed paralysis and death time in 7, 6 and 36, 28 mins respectively. The effect increased with concentration. The extract caused paralysis followed by death of earthworms at all tested dose levels.

Conclusion: The extract showed paralysis followed by death of worms at all tested dose levels. Based on the study the Ethanolic leaf extract of *Cardiospermum halicacabum* has potent anthelmintic activity.

Key Words: Albendazole, Paralysis time, Death time, Helminthiasis, Indian Earth worm, Physiological Damage

INTRODUCTION

Macro parasitic disease of individuals and other creatures in which the portion of the body infected with parasitic worms called as Helminths¹. It is otherwise known as intestinal worm infestation, helminthic infestation or soil transmitted infestations. The parasitic worm are invertebrates characterize extended, smooth, or rounded forms. These worms get burrowed into the organs or gastrointestinal tract of host and induces a physiological damage. The different kind of worms consists of strongloidiasis, trichinella, blood flukes, lymphatic filariasis, Hookworm, Flatworm and Tapeworm.

Female produces eggs (almost 200000 each day) that remain defaecated with feces. Unfertilized eggs remain in offensive, but fertilized once are infectious after 18 days to several weeks.

Infectious eggs are consumed and arrive in the gut. Mature

into larvae in the intestine, and penetrate the blood vessels to enter lungs, where they develop further. At day14, they penetrate into the alveolar walls. As they reaching the small lintestine and develop into adultworm.

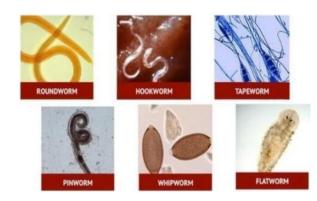


Figure 1: Types of worms².

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ISSN: 2231-2196 (Print) **ISSN:** 0975-5241 (Online)

LIFECYCLE OF HELMINTHS

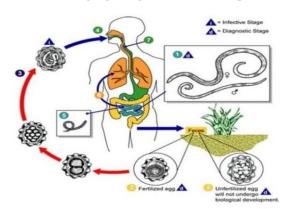


Figure 2: Lifecycle of Helminths³.

INDICATIONS

For minor infections, no evident symptoms are seen. For long and heavy infections, a variety of health complications are anemia, malnutrition, growth retardation, developmental retardation, intestinal obstruction, decreasing protein loss and blood loss, less cognitive and physical growth, inability to control motion. People with light infection usually have no symptoms.

TRANSMISSION

Soil-transmitted Helminths are spread by eggs that are passed in the feces of infected persons. Eggs that are attached to vegetables are ingested when the vegetables are not carefully cooked, washed or peeled. Hook worm egg hatch in the soil releasing larvae that mature into a form that can actively penetrate the skin. There is no direct person to person transmission or infect from fresh feces because eggs passed in feces need about 3 weeks to mature in the soil before they become infective.

DIAGNOSIS

Some of the diagnostic methods are Microscopy-based techniques, Eggs counting Kato- Katz method, Serological assays, Endoscopy/Colonoscopy, X-ray, Magnetic Resonance Imaging (MRI) scan, Computerized Axial Tomography scan(CAT)⁴

PREVENTION

Avoid interaction with soil contaminated with human waste especially feaces. Wash hands thoroughly with soap before taking food. Infection will be prevented by disrupting the cycle of the worm. And other preventive measures are by sanitation and hygiene, provision of safe drinking water, wearing protective footwear. Not evacuating outside use toilets linked to current sewage discarding systems.

TREATMENT

Various allopathic drugs are use in the treatment of Helminthiasis. Benzimidazoles (albendazole, mebendazole, thiabendazole), piperazine citrate, pyrantel pamoate, levamisole, Tetraisole, Diethylcarbamazine citrate (dec), Ivermectin, Praziquantel, Niclosamide ⁴

HERBALMEDICINE

Though Allopathic drugs produce the onset of action quick, it produces many adverse effects. So, now a days herbal drugs are preferred mostly when compared to that of allopathic drugs because It does not produce side effects. Some of the most widely used herbal drugs are as follows,

Table 1: List of some herbal medicinal plants with anthelmintic activity

| S. NO | BotanicalName | Family | Common Name | Parts Used |
|-------|---------------------|----------------|------------------------|--------------------|
| 1 | Calotropisgingantea | Asclepiadaceae | Crown flower | Roots |
| 2 | Vitexnegundo | Lamiaceae | Chinese Chaste Tree | Leaves and flowers |
| 3 | Cleomeviscose | Capparidaceae | Asian Spider Flower | Seeds |
| 4 | Helicteresisora | Malvaceae | East Indian Screw Tree | Bark |
| 5 | Terminaliachebula | Combretaceae | Chebulic Myrobalan | Fruits |
| 6 | Benincasahispida | Cucurbitaceae | White gourd | Roots |
| 7 | Caesalpiniabonduc | Fabaceae | Grey nicker | Stem |
| 8 | Neolamarckiacadamba | Rubiacea | Burflower Tree | Bark |
| 9 | Curcumacaesia | Zingiberaceae | Turmeric | Rhizome |
| 10 | Sophorainterrupta | Fabaceae | Adavibillu | Leaves |
| 11 | Trachyspermumammi | Apiaceae | Bishopsweed | Seeds |

Table 1: (Continued)

| S. NO | BotanicalName | Family | Common Name | Parts Used |
|-------|--------------------------|---------------|-----------------|------------|
| 12 | Coriandrumsativum | Apiceae | Chinese Parsley | Leaves |
| 13 | Artocarpus heterophyllus | Moraceae | Jackfruit | Seeds |
| 14 | Semecarapusanacardium | Anacardiaceae | Marking nut | Seeds |
| 15 | Buteamonosperma | Fabaceae | Flame of forest | Flower |

MATERIALS AND METHODS

Collection of plant:

Cardiospermum halicacabum leaves were collected from Chennai. The leaves were identified, confirmed and authenticated from Siddha Central Research Institute, Arumbakkam, Chennai with reference No:117.05021903 by comparing with an authentic specimen by a botanist.

Preparation of extract:

Percolation is an extraction process that involves the slow descent of a solvent through a powdered substance until it absorbs certain constituents and drips out through the filtered bottomof the container. Percolation can be done by using the Soxhlet apparatus. ⁶



Figure 3: Working of Soxhlet apparatus⁷

Phytochemical analysis

For our present study, preliminary phytochemical studies were carried out to characterize the therapeutic active constituent from the leaf extract of *Cardiospermum halicaca*^[8]

Screening of anthelmintic activity by *In-Vitro* method

Indian earthworms (*Pheretima posthuma*) were collected from water-logged area of soil. The average size of

each worm collected is about 14-16 cm length. They were washed thoroughly with tap water to remove the adhering dirt. Five Sterile Petridishes were taken. In each Petridish 6 earthworms were placed and kept in a room temperature. Distilled water was used as a control. Distilled water was poured into Petridish containing 6earthworms (Group-1).

Albendazole, the standard drug was diluted with distilled water to obtain 10mg/ml concentrationand then it was poured into Petri dish containing 6 earthworms. (Group-2) Ethanolic extract of *Cardiospermum halicacabum* was diluted with distilled water to obtain 100mg/ml, 200mg/ml,300 mg/ml concentrations and it was poured into Petri dish containing 6 earthworms(Group-3,4,5 respectively The time taken for paralysis(when there was no movement of worms) were observed and the time taken for death(when there is no movement on vigorous shaking also) was noted. It was noted in terms of minutes.^{8,9,10}

STATISTICAL ANALYSIS

Values are expressed as mean \pm SEM. One-way ANOVA followed by Dunnett's test to assess the statistical significance.

RESULTS

Preliminary Phytochemical studies

The result of preliminary phytochemical analysis of the Ethanolic leaf extract of *Cardiospermum halicacabum* shows the presence of Flavonoids, Glycosides, Tannins, Aminoacids, Steroids, Phenols, Saponins, Carbohydrates.¹¹

Anthelmintic activity^{12,13,14}

The anthelmintic activity of *Cardiospermum halicacabum* was analyzed by in vitromethod by using the Indian earthworms. The activity was performed and the paralysis time and death time was noted down. The anthelmintic activity of the extract was compared with that of the standard drug Albendazole.¹⁵

Table 2: Paralysis and death time (minutes) of *Pheretima posthuma* at different concentrations 8

| TREATMENT | CONCENTRATION S (mg/ml) | PARALYSISTIM E (min) | DEATHTIM E (min) |
|--------------------------------------|----------------------------|-------------------------|---------------------|
| CONTROL | - | - | - |
| STANDARD (Albendazole) | 10(mg/ml) | 4.71±0.20*** | 26.25±0.37** |
| TEST (Cardiospermum Halicacabum) | 100(mg/ml) | 14.93±0.16** | 44.52±0.30** |
| TEST (Cardiospermum halicacabum) | 200(mg/ml) | 8.86±0.27*** | 34.79±0.21** |
| TEST (Cardiospermum halicacabu m) | 300(mg/ml) | 5.71±0.44*** | 27.98±0.41*** |

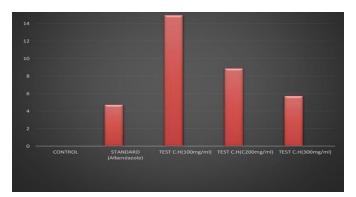


Figure 4: Graphical representation for the anthelmintic activity of ethanolic extract of *Cardiospermum halicacabum* (100mg/kg) showed higher concentrations compared to the standard drug (Paralysis time).



Figure 5: Graphical representation for the anthelmintic activity of ethanolic extract of *Cardio spermum halicacabum* (100mg/kg) shows higher concentrations compared to the standard drug (Death time).

EFFECT OF DIFFERENT CONCENTRATIONS OF DRUGS ON PHERETIMAPOSTHUMA



Figure 6: Distilled water.



Figure 7: Albendazole 10mg/ml.



Figure 8: Cardiospermum halicacabum 100mg/ml.



Figure 9: Cardiospermum halicacabum 200mg/ml.



Figure 10: Cardiospermum halicacabum 300mg/ml.

DISCUSSION

The anthelmintic activity of leaves extract of *Cardiospermum halicacabum* was carried out on earthworm. Different concentrations of the ethanolic extracts were used for the studies. The time taken for paralysis and death of earthworms were recorded in table 1. The perusal of the data reveals that the ethanolic extract at the concentrations of 100 mg/ml, 200 mg/ml, 300mg/ml showed paralysis and death time in 7,6 and 36, 28 mins respectively. The effect increased with concentration. The extract caused paralysis followed by death of earthworms at all tested dose levels. It was observed that the ethanolic extract of *Cardiospermum halicacabum* is more potent drug. Extract exhibited paralysis followed by death of worms at all established dose levels. The extract potency was found to be inversely proportional to the time taken for paralysis of death of worms.

CONCLUSION

From the present study we concluded that the preliminary photochemical analysis of the Ethanolic leaf extract of *Cardiospermum halicacabum* shows the presence of Flavonoids, Glycosides, Tannins, Aminoacids, Steroids, Phenols, Saponins, Carbohydrates. The Ethanolic leaf extract of Cardiospermum halicacabum has anthelmintic activity.

ACKNOWLEDGEMENT

We wish to express sincere gratitude to Dr. Meena Principal, Dr. Shanthi Vice-Principal, K.K. College of Pharmacy for the support and encouragement towards our research work.

Conflict of interest: None

Source of Funding: None

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