RESEARCH ARTICLE

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Evaluation of Antihelminthic Activity of Hydroalcoholic Extract of Anthocephalus Cadamba

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ABSTRACT

Aim: This research aimed to evaluate the anthelmintic activity of a hydroalcoholic extract of *Anthocephalus cadamba (HEAC)* leaves using *Pheretima posthuma* as a model organism, thereby assessing its potential as a natural therapeutic agent against parasitic infections.

Methodology: The plant material was sourced from Shadnagar, verified, and then macerated to extract the active ingredients. Phytochemical analysis identified phenolic substances, alkaloids, saponins, and flavonoids. Tests for anthelmintic activity were conducted on five groups of Indian earthworms (Pheretima posthuma), with six worms in each group. Group I was given normal saline, Groups II-IV were given hydroalcoholic extracts at concentrations of 25, 50, and 100 mg/mL, whereas Group V was given 200 mg/mL of albendazole. We used one-way ANOVA ($P < 0.05$) to examine the data, which included recording the timings of paralysis and death.

Results: Bioactive chemicals including alkaloids, saponins, flavonoids, and phenolic compounds were identified in Anthocephalus cadamba leaves by a phytochemical study. The hydroalcoholic extract showed anthelmintic action that was dosage-dependent. When compared to 25 mg/mL and 50 mg/mL concentrations, the 100 mg/mL dose exhibited significantly greater activity. This proved that the extract was effective in paralyzing and killing the earthworms since its activity was similar to that of the reference medicine Albendazole.

Conclusion: The hydroalcoholic extract of *Anthocephalus cadamba* leaves exhibits strong, dose-dependent anthelmintic activity, likely due to its phytochemical constituents. Further research is required to optimize extraction methods and evaluate the extract's efficacy in clinical models. Nonetheless, this study supports its potential use as a natural anthelmintic agent.

Keywords: *Pheretima posthuman, Anthocephalus cadamba,* Anthelmintic activity, Albendazole, Maceration.

1. INTRODUCTION

Parasitic worms, such as tapeworms (cestodes), roundworms (nematodes), and flukes (trematodes), cause helminthiasis, or worm infection.[1] Although these parasites most often inhabit the digestive system, they are capable of invading other organs and causing serious physiological harm if they do so. While helminth infections manifest in different ways in different parts of the body, the persistence and influence of these parasites originate from their intricate multicellular structures, which include muscular, neurological, digestive, and reproductive systems.

Roughly 25–33% of the population in underdeveloped nations suffers from helminthiasis; in regions with severe poverty and inadequate sanitation, the incidence rate may reach 90%.[2] Factors that increase the likelihood of acquiring these illnesses include a lack of proper sanitation, water contamination, improper hygiene habits, excessive population density, and a lack of educational and medical facilities. The frequency of soil-transmitted helminths (STHs) such Ascaris lumbricoides and Trichuris trichiura is worse in high-risk zones because these parasites enter humans via contaminated soil, and hookworm larvae may directly permeate skin.[3]

While helminth infections have far-reaching consequences on human health and development, they are often disregarded and thus are considered neglected tropical illnesses.[4] These illnesses have the potential to slow development, lower output, and keep people

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mired in poverty. There is a marked lack of investment in tackling helminthiasis, a public health crisis that affects people all over the world, as research into this disease gets less than one percent of the total research budget.

The main course of therapy for helminth infections is anthelmintic medication. Paralysis or energy depletion may occur as a result of these drugs' effects on the parasite's nervous system or energy metabolism, which are impaired due to impaired glucose absorption.[5] Nevertheless, there are limits to the current therapies that need to be addressed. No anthelmintic has been shown to be 100% effective, non-toxic, or capable of eliminating all helminths. These difficulties highlight the significance of maintaining a research and development strategy focused on developing safer and more effective treatment choices. As opposed to synthetic medications, herbal alternatives are more cost-effective and often have fewer unwanted side effects. Thus, the purpose of this research is to test A. cadamba's antihelminthic effectiveness against P. posthuma with albendazole serving as a positive control.

The tropical tree A. cadamba, often called the burflower tree or kadamba, is a member of the Rubiaceae family. Among its many medicinal uses are the alleviation of fever, leprosy, diarrhea, blood, and skin disorders. Additionally, it possesses hepatoprotective, wound-healing, and antioxidant capabilities. Ayurvedic practitioners have long recognized the therapeutic benefits of the plant Anthocephalus cadamba, which include relief from diarrhea, detoxification, pain, and seminal fluids.[6-9]

2. METHODOLOGY

2.1 Collection of anthocephalus cadamba

Plant samples were collected from Shadnagar. The Department of Botany at Osmania University in Hyderabad, Telangana, headed by Dr. A. Vijaya Bhasker Reddy, verified its authenticity.

2.2 Experimental animal models

The experiment used Pheretima posthuma (of around the same size), which are brown earthworms native to India. The Maillardevpally dairy farm was the source of their collection.

2.3 Grouping of animals

Earthworms were divided into five groups, and each group had six of them. The control group was Group I. The hydroalcoholic extract was administered to Groups II, III, and IV at doses of 25, 50, and 100 mg/mL, correspondingly. The fifth group received 200 mg/mL of the standard medication albendazole (Figure 1).

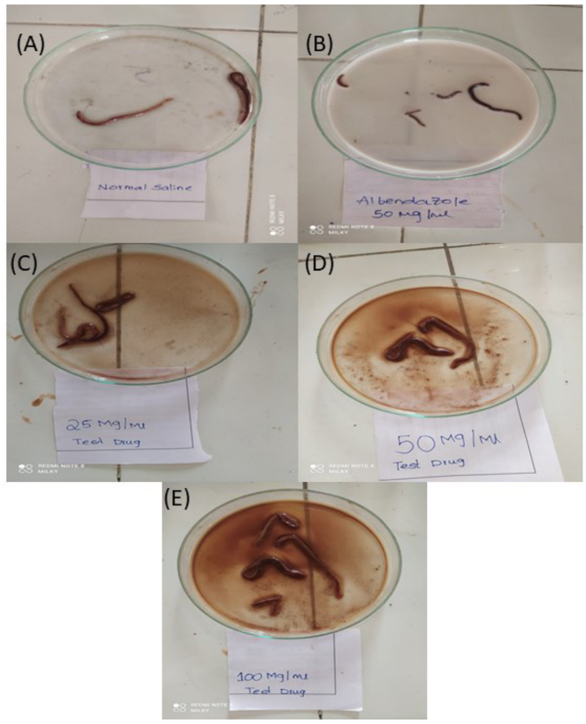

Figure 1: Grouping of worms. (A) Control group, (B) Albendazole 50mg/mL, (C) Ethanolic extract (25mg/mL), (D) Ethanolic extract (50mg/mL), (E) Ethanolic extract (100mg/mL)

2.4 Method of extraction

The extraction method used in our investigation was maceration. A solvent was selected for the extraction of the necessary bioactive components, and then coarsely powdered plant material was immersed in it. To maximize the plant material's surface area and facilitate its contact with the solvent, it was crushed into smaller particles.

To enhance extraction, the solvent-plant combination was let to stand for a long time with occasional stirring. Then, in order to isolate the extract, the mixture was filtered via a suitable filtering media. Both the plant material's characteristics and the solvent employed to extract it have an impact on the process's efficiency. Reasons for using this technology include its ease of use, low cost, and capacity to produce the desired bioactive chemicals on a laboratory scale.

2.5 Phytochemical Screening :

Test for alkaloid

- Mayers reagent - Hgcl & KI2 in 100 mL water
- Appearance of yellow cream ppt – Result: Positive

Test for carbohydrates

Molish's reagent - Alpha naphthol in 25 mL of ethanol subsequent addition of a few drops of concentrated sulphuric acid

- Formation of violet ring Result: Positive

Test for saponins

- Fehling's reagent – Fehling's B clear liquid in Rochelle salt (potassium sodium tartrate) + strong alkali NaoH
- Formation of red precipitate Result: Positive

Test for phenol

- Ferric chloride test - organic solvent in water + ferric chloride dropwise.
- Appearance of bluish-black precipitate Result: Positive

Test for flavonoids

- Lead Acetate Test - Extract a few drops of lead acetate.
- Yellow precipitate obtained Result: Positive

Test for fats and oils

- Solubility test - The sample was dissolved in ether, chloroform, and ethanol and observed for solubility.
- Result: Negative

2.6 Invitro study of antihelmintic activity

2.6.1 Collection of worms

An anthelmintic activity investigation was conducted on Indian earthworms in April 2023 using worms gathered from a dairy farm close to Maillardevpally. These earthworms were selected to test the effects of anthelmintics because they are physiologically and anatomically comparable to human intestinal roundworm parasites. For the experiment, we used earthworms that were between 0.1 and 2 centimeters wide and 3 to 5 centimeters long.

2.6.2 Antihelminthic activity method

Earthworms with uniform dimensions were chosen for the anthelmintic activity investigation and assigned to one of five groups, with six worms per group. By combining the extract with distilled water and adjusting the final quantities as needed, hydroalcoholic extracts were generated with varying strengths (25, 50, and 100 mg/mL). Both the extracts and the reference medicine were ready for evaluation.

The activity was evaluated after adding the standard solution and extracts at different concentrations to different Petri plates. The worms' paralysis and death times were recorded by careful observation. The average duration of paralysis and death for each group was subsequently recorded.

The paralysis time was defined as the duration it took for the worms to stop moving, while the lethal time was determined by timing how long it took for the worms to stop moving after being pricked with a pin. The medicine albendazole was used as the standard for comparison.

2.7 Statistical Analysis

When comparing multiple groups, statistical analysis was performed using GraphPad Prism 5 and one-way analysis of variance (ANOVA) followed by Tukey's test. If the P-value was less than .05, the differences were deemed statistically significant.

3. RESULTS AND DISCUSSION

3.1 Preparation of extract

The bioactive components were successfully isolated from the plant material during the extraction process. An efficient method for processing coarsely powdered leaves, stem bark, and root bark was to use a menstruum, which completely submerged the material. With regular shaking and stirring, the extraction process was finished after three days, allowing for the comprehensive release of the compounds (Figure 2).

The marc and micelle were effectively separated by the filtering or decantation procedure. The menstruum was removed by further evaporation of the micelle in an oven or water bath, leaving behind the required plant extract (Figure 3). This approach produced a high-quality extract with minimum degradation, which was especially useful for thermolabile plant components.

Analyses of the phytochemical composition of Anthocephalus cadamba leaf powder have shown the presence of phenolic compounds, saponins, flavonoids, and a high alkaloidal content (Table 1).

Figure 2: Plant extraction using maceration

Table 1: Phytochemical screening of the extract

S. No.	*Compound*	*Test*	*Ethanolic extract*
1	Test for Alkaloids	Mayers	+
2	Test for carbohydrates	Molish's	+
3	Test for saponins	Fehling's	+
4	Test for phenol	Ferric chloride	+
5	Test for flavanoids	Lead acetate	+
6	Test for Resins	Acetone water	-

Table 2: Antihelminthic activity of *A. cadamba*

S. No.	*Treatment of Groups*	*Time taken to complete paralysis (min) Mean ±S.E.M*	*Time taken for death (min) Mean ±S.E.M*
1	Control normal saline	>60	>60
2	Anthocephalus cadamba (25 mg/mL)	28 ± 1.5*	34 ± 1.7*
3	Anthocephalus cadamba (50 mg/mL)	25 ± 2.2*	29 ± 1.9*
4	Anthocephalus cadamba (100 mg/mL)	8.7 ± 1.1	10 ± 0.84*
5	Albendazole (50 mg/mL)	2.2 ± 0.48	4.2 ± 0.31*

Figure 3: Drying of the plant extract

Table 2 shows that the findings revealed a dose-dependent paralytic effect that occurred between the time of administration and the time of death. Results indicated that paralysis and death occurred more quickly at greater extract concentrations. The extracts demonstrated significant anthelmintic activity ($P < .05$) in comparison to the standard. In contrast to the reference medicine Albendazole, which exhibited paralysis and death times of 2.2 and 4.2 minutes, respectively, the extract at 100 mg/mL caused them at 8.7 and 10 minutes, respectively.

Results are expressed as mean ± SEM. N=3; $*P < .05$, as compared to standard.

4. DISCUSSION

Since Indian adult earthworm (P. posthuma) is physiologically and anatomically similar to human intestinal roundworm parasites, all of the experiments in this work were conducted in vitro on this species. Infectious diseases caused by these parasite helminths are a constant problem for both humans and animals. While there have been several advancements in the development of synthetic chemicals and their derivatives, the use of these treatments is not without its issues, as they may cause major adverse effects. Additionally, parasites might become resistant to medications, which can worsen illnesses. The development of herbal medications as an alternative, less harmful treatment for helminths has therefore been initiated. In this experiment, we have assessed the anthelmintic efficacy of A. cadamba at concentrations of 25, 50, and 100 mg/mL. The study's findings indicate that anthelmintic activity has a promising future. It is possible that this plant might help humans reduce nematode infections in the intestines. The results show that the time it takes for albendazole to cause paralysis and death is comparable to the time it takes for HEAC to cause paralysis and death. These findings are consistent with those of other research.[10-12]

Analyses of the phytochemical composition of Anthocephalus cadamba leaf powder have shown the presence of phenolic compounds, saponins, flavonoids, and a high alkaloidal content. Previous research has shown that phenolics kill helminth parasites due to their prooxidant activity or by interfering with their energy generation processes.[13] Flavonoids and alkaloids, on the other hand, are responsible for their anthelmintic activity.[14,15] To determine the pharmacological effectiveness of A. cadamba as an anthelmintic medicine, further research using in vivo models is required.

5. CONCLUSION

Using Indian earthworms as a model, the research successfully proved the anthelmintic activity of A. cadamba extract. Earthworms were found to be paralyzed and eventually die when exposed to the extracts at different concentrations; the exact duration of paralysis and death was noted for each group. The reference medicine albendazole was used to compare the extracts' activities. This study lends credence to the idea that A. cadamba may have future application as a powerful anthelminthic agent in the fight against helminths that have developed resistance. Additional research is needed to better understand the phytochemical profile, isolate the bioactive components, and identify their role in anthelminthic action.

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