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Original Research Article

In Vitro Evaluation of anthelmintic activity of *Lactuca Sativa* leaf extract against Pheretima posthuma

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ABSTRACT

The growing issue of anthelmintic resistance necessitates the exploration of alternative and natural treatments. Lactuca sativa (*L. sativa*), commonly known as lettuce, is traditionally used for its medicinal properties, but its efficacy as an anthelmintic has not been extensively documented. This study aimed to assess the in vitro anthelmintic activity of *L. sativa* leaf extract using Indian adult earthworms. Earthworms were divided into five groups and treated with hydroethanolic extract of *L. sativa* leaf at concentrations of 10, 20, and 50 mg/mL, albendazole 10 mg/mL as a standard, and normal saline as a control. The anthelmintic activity was measured based on paralysis and death times. The results demonstrated that the anthelmintic activity of the extracts was concentration-dependent manner. The extract 50 mg/mL exhibited superior activity with a paralysis time of 28.16 minutes and a death time of 36.33 minutes, compared to albendazole, which had a paralysis time of 24.50 minutes and a death time of 31.66 minutes. The study concluded that the hydroethanolic extract of *L. sativa* leaf (HELS) possesses significant anthelmintic activity against Indian adult earthworms.

1. Introduction

Helminth infections are among the most prevalent diseases affecting humans globally. While these infections are widespread, they are particularly common in tropical regions, where they pose significant health risks. These parasitic diseases can lead to a range of complications, including anemia, imbalances in the body's systems, eosinophilia, and pneumonia.[1] Helminthiasis primarily results from the infestation of the human body by worms such as pinworms, tapeworms, or roundworms. These parasites often inhabit not only the gastrointestinal tract but can also migrate to other organs, including the liver. Infected individuals release helminth eggs in their feces, which subsequently contaminates the soil, particularly in areas with inadequate sanitation.[2] Gastrointestinal helminths have shown potential resistance to the currently available anthelmintic drugs, creating a significant challenge in effectively treating helminth infections.[3]

Lactuca sativa, commonly known as garden lettuce, is an annual leafy vegetable from the Asteraceae family. This versatile and widely cultivated plant is noted for its milky sap, which contains lactucarium. Lactucarium has been traditionally used for its sedative, digestive, diuretic, and narcotic properties.[4,5] In traditional medicine, *L. sativa* has been employed to address stomach issues, stimulate digestion, enhance appetite, and reduce inflammation.[6] Pharmacological research has demonstrated the plant's potential therapeutic benefits across various studies. However, its anthelmintic activity has not yet been investigated. Consequently, this study aims to evaluate the anthelmintic potential of *L. sativa* leaf extract against Indian adult earthworms.

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2. Materials and Methods

2.1. Plant material

Lactuca sativa plant was collected from Latur, Maharashtra, India. The plant materials were authenticated by the Botanical Survey of India (Reference No. BSI/WRC/Tech./2024/JVD-43).

2.2. Drugs and chemicals

All organic solvents and chemicals were purchased from Labware Chemical Pvt. Ltd., Latur and were of analytical grade.

2.3. Preparation of crude extract

The leaf of *L. sativa* was thoroughly rinsed with tap water and distilled water, then placed in a shaded area to dry. Once dried, the material was coarsely ground with a mortar and pestle, then finely powdered using an electric blender, and stored in an airtight glass container. To prepare the extract, 20 grams of the powdered material were subjected to solvent extraction with 100 mL of hydroethanolic solvent for 5 hours. The resulting hydroethanolic extract was then dried and used to evaluate anthelmintic activity.

2.4. Phytochemical screening

The phytochemical analysis of the extract was performed using standard procedures.[7] The leaves of *L. sativa* were specifically analyzed to identify and detect the major chemical constituents.

2.5. Earthworms' collection

The anthelmintic activity was assessed using P. posthuma earthworms, which were collected from Latur, Maharashtra, India. The worms were thoroughly washed with normal saline to remove any fecal matter. Earthworms measuring 4-5 cm in length and 0.1-0.2 cm in width, weighing between 0.8 and 3.04 grams, were used for the experiments. These earthworms were chosen due to their anatomical and physiological similarity to human intestinal roundworm parasites, making them suitable for studying anthelmintic activity.[8]

2.6. Preparation of test drug and reference drug

For the in vitro study, HELS were prepared at concentrations of 10, 20, and 50 mg/mL. Hydroethanolic extracts were prepared by dissolving 100, 200, and 500 mg of crude extract in minimum quantity of 2% v/v Tween 80 and adjusted the final volume of 10 mL with normal saline solution. This resulted in final concentrations of 10, 20, and 50 mg/mL, respectively. Normal saline was used as the control, and albendazole was employed as the standard drug for comparison.[9]

3. Anthelmintic Activity

Anthelmintic activity was assessed using the method described by Hussain et al., with concentrations of 10, 20, and 50 mg/mL against Indian earthworms (P. posthuma).[10] Five groups of Indian earthworms, each consisting of six worms of approximately equal size, were used in the study. Three groups were treated with extracts at concentrations of 10, 20, and 50 mg/mL, one group was treated with albendazole at 10 mg/mL as the reference standard, and one group served as the control, receiving normal saline solution. The anthelmintic activity was evaluated by observing the time required for paralysis and death of the earthworms.

3.1. Statistical analysis

All results are expressed as mean ± SEM. Data was analyzed using ANOVA followed by Dunnett's multiple comparison test. Statistical analysis was performed with GraphPad Instat Software (Version 3, USA). Results were considered statistically significant when $P < 0.01$.

4. Results

Preliminary phytochemical screening of the extract identified the presence of alkaloids, phenols, tannins, flavonoids, carotenoids, terpenoids, and steroids. The extract induced a dose-dependent paralysis in earthworms, ranging from loss of motility to unresponsiveness to external stimuli, ultimately leading to their death. Table 1 shows the anthelmintic activity of HELS at different concentrations, compared with the reference standard albendazole. At concentrations of 10, 20, and 50 mg/mL, HELS produced paralysis times of 70.83, 51.83, and 28.16 minutes, and death times of 76.33, 57.83, and 36.33 minutes, respectively. The highest concentration showed a rapid onset of paralysis and death comparable to the reference standard albendazole. For comparison, the albendazole treated group at 10 mg/mL had a paralysis time of 24.50 minutes and a death time of 31.66 minutes. In the control group (normal saline), the worms were observed for 24 hours with no signs of paralysis or death (Figure 1).

5. Discussion

The anthelmintic activity of *L. sativa* was evaluated against Indian adult earthworms (P. posthuma) and demonstrated a significant, dose-dependent effect ($P < 0.01$). Albendazole, used as a reference standard, primarily caused flaccid paralysis in the worms, leading to their expulsion through peristalsis. Albendazole works by binding to free β-tubulin, inhibiting its polymerization, and thereby disrupting microtubule-dependent glucose uptake in the worms. It exhibits a selective inhibitory effect on helminth microtubular function, being 300-400 times

Table 1: Anthelmintic activity of *L. sativa* leaf extract

Treatment	Concentration(mg/mL)	Time taken in minutes (mean ± SEM)	
		Paralysis	Death
Control	Normal Saline	——	——
Albendazole	10	24.50 ± 0.96	31.66 ± 0.76
HELS	10	70.83 ± 0.65**	76.33 ± 1.05**
HELS	20	51.83 ± 0.87**	57.83 ± 1.19**
HELS	50	28.16 ± 0.54**	36.33 ± 0.88**

**: P<0.01. Values are in mean±SEM, n=6, when compared with albendazole. Values of P<0.01 were considered as statistically significant.

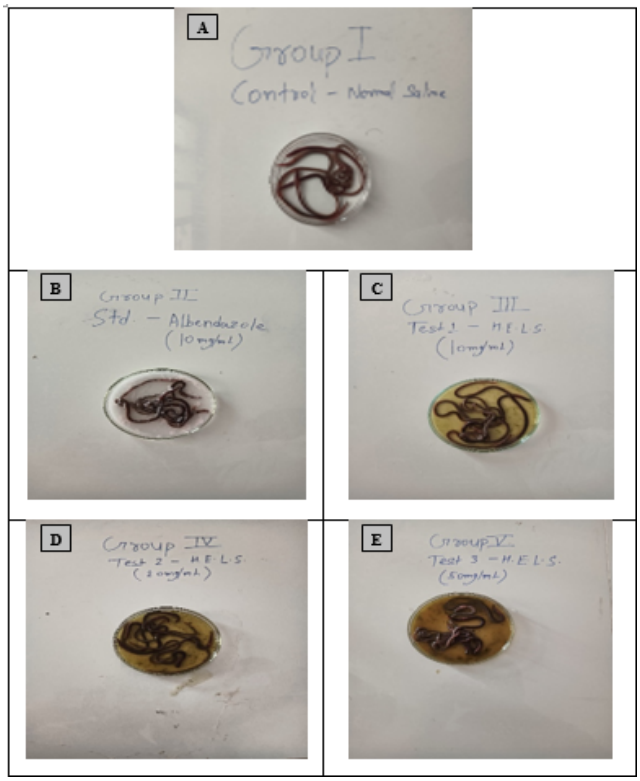

Figure 1: Anthelmintic activity of *L. sativa* leaf extract; **A:** Normal Saline, **B:** Albendazole 10 mg/mL, **C:** HELS 10 mg/mL, **D:** HELS 20 mg/mL and **E:** HELS 50 mg/mL.

more potent against helminths than against mammalian tissues.[11] *L. sativa* leaf extract contains different secondary metabolites that contribute to its anthelmintic activity. Preliminary phytochemical screening of *L. sativa* identified the presence of alkaloids, phenols, tannins, flavonoids, carotenoids, terpenoids, and steroids. Notably, tannins, which are polyphenolic compounds, have been documented to exhibit anthelmintic properties.[12] Certain synthetic phenolic anthelmintics, such as niclosamide, oxyclozanide, and bithionol, are known to disrupt energy production in helminth parasites by uncoupling oxidative phosphorylation.[13] It is possible that the tannins present in the *L. sativa* extract may exert similar effects by interfering with energy production in helminths. Additionally, tannins might contribute to anthelmintic activity by binding to free proteins in the gastrointestinal tract of the host animal, which could lead to the death of the parasites.[14,15] Alkaloids are known to induce paralysis by acting on the central nervous system.[16] Therefore, it is plausible that the anthelmintic activity of *L. sativa* may be due to the presence of phytochemicals such as tannins and alkaloids. The findings from this bioassay support the traditional use of *L. sativa* for its anthelmintic properties. However, further research is necessary to isolate and characterize the specific bioactive components and elucidate their mechanisms of action.

6. Source of Funding

None.

7. Conflict of Interest

None.

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