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# EVALUATION OF ANTHELMINTIC EFFICACY OF 50% HYDROETHANOLIC LEAF EXTRACT OF HYDROCOTYLE VERTICILLATA THUNB. IN ADULT AFRICAN EARTHWORMS

Biochemistry

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## ABSTRACT

Helminthiasis remains one of the most widespread parasitic infections globally, representing a significant public health challenge. The present study aims to evaluate the anthelmintic activity of a 50% hydroethanolic leaf extract of Hydrocotyle verticillata Thunb. against adult African earthworms (Eudrilus eugeniae) under in vitro conditions. The earthworms were divided into four groups, with six worms in each group. The anthelmintic activity of the hydroethanolic extract of H. verticillata was assessed at two concentrations (25 mg/mL and 50 mg/mL) by recording the time to paralysis and time to death. Albendazole (25 mg/mL) served as the standard reference, while distilled water was used as the control. The lower dose of the extract produced paralysis and death at 43.31 and 48.39 minutes, respectively, while the higher dose reduced these times to 27.24 and 32.80 minutes, demonstrating clear dose-dependent efficacy. Both concentrations of the plant extract showed measurable activity, with the higher dose exhibiting results comparatively closer to the reference drug. These findings highlight the promising anthelmintic potential of H. verticillata, supporting the need for further studies at higher concentrations to establish its effectiveness and therapeutic relevance.

## KEYWORDS

Helminthiasis, Hydrocotyle Verticillata, Antihelmintics, Eudrilus Eugeniae

## INTRODUCTION

Helminthiasis is a parasitic disease caused by worms such as pinworm, roundworm, and tapeworm, primarily affecting the gastrointestinal tract [1]. Helminth infections are among the most common afflictions in humans, impacting a large proportion of the global population. In developing countries, they pose a major public health burden and contribute significantly to malnutrition, anaemia, and eosinophilia etc., Although helminthiasis is rarely fatal, it remains a leading cause of morbidity worldwide [2]. According to the World Health Organization (2023), an estimated 1.5 billion people - approximately 24% of the world's population—are affected by helminth infections [3].

The control of these helminth infections depends on a narrow range of available anthelmintic drugs. A large number of commercially available drugs such as albendazole, mebendazole, levamisole, and pyrantel pamoate are frequently employed to treat helminth infections [4]. While effective, these drugs are associated with several drawbacks, including adverse effects such as headache, nausea, vomiting, diarrhoea, abdominal discomfort, dizziness, and vertigo [5]. Long-term use may also affect vital organs such as the liver and kidney [6]. Moreover, prolonged therapy has led to the emergence of drug resistance in helminth parasites [7]. These challenges emphasize the necessity for the discovery and development of new anthelmintics with innovative mechanism of action.

Considering the limitations of existing drugs and their high cost, medicinal plants are increasingly being explored as promising sources of novel anthelmintic agents. Plants are rich in secondary metabolites that have been used in traditional medicine for centuries to treat a wide range of diseases [8]. In many developing countries, a large proportion of the population continues to depend on herbal remedies as their primary source of healthcare. The global use of herbal medicines is steadily increasing, underscoring the importance of thorough biological evaluation of plant extracts to ensure their efficacy and safety [9].

*Hydrocotyle verticillata* Thunb., commonly known as water pennywort, belongs to the family Araliaceae and is widely distributed across South and North America and the West Indies. Traditionally, different parts of the plant have been employed in folk medicine for a variety of ailments. Its juice has been used to reduce fevers, while poultices prepared from the leaves are applied to wounds, boils, and skin ailments. Decoctions are administered for abscesses, coughs, colds, hepatitis, influenza, pruritus, and sore throat. In China, the plant is prescribed for hepatoma and hepatitis and incorporated into herbal formulations for muscular dystrophy. In India, preparations of the juice mixed with honey, are consumed for typhoid fever and febrile conditions [10, 11]. Previous studies have reported the presence of saponins and novel phytochemicals with antitumor, immunomodulatory, antioxidant, and antiproliferative activities, along with compounds beneficial to liver health [12]. These findings support its ethnomedicinal relevance and highlight the need for systematic biological evaluation to validate traditional claims and explore its pharmacological potential.

## Materials and methods

### Plant collection and authentication

The leaves of *Hydrocotyle verticillata* Thunb. were collected from local areas in Hosur. The plant material was authenticated by Dr. Soosairaj, Associate Professor, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu. A reference specimen has been deposited in the Department of Botany, St. Joseph's College, Tiruchirappalli, under the accession number 8315.

### Preparation of the dry powder

The fresh leaves of *Hydrocotyle verticillata* Thunb was thoroughly washed using running tap water to remove soil and dried by shade drying method at room temperature for a week. The dried leaves were ground to a coarse powder and used for the preparation of the extract.

### Preparation of 50 % hydroethanolic extract

About 250 g of *Hydrocotyle verticillata Thunb* leaves were immersed in 500 ml of 50% hydroethanol and subjected to cold maceration for 72 hours. The mixture was stirred intermittently during the extraction period. After extraction, the suspension was filtered through fine muslin cloth, and the resulting filtrate was concentrated on a water bath until dryness. The process yielded dark brown crystalline residues, which were stored in airtight containers. The dried extract was re-dissolved and used for subsequent study.

### Worm collection

Adult African earthworms *Eudrilus eugeniae* (African nightcrawler) of nearly 12 – 14 cm ± 2 were purchased from Arun Vermitech Industry, Bagalur, Hosur. The earthworms were maintained in moist, well aerated soil at room temperature in the Biochemistry Laboratory in a wooden box.

### Anthelmintic activity of *Hydrocotyle verticillata*

The anthelmintic potential of *Hydrocotyle verticillata* Thunb. was assessed using *Eudrilus eugeniae* earthworms, chosen for their anatomical and physiological similarity to human intestinal roundworms. Earthworms were collected from moist soil, thoroughly rinsed with normal saline to remove debris, and then used for the in vitro assay following a slightly modified protocol described by Ajaiyeoba *et al.,* 2001 [12].

The worms were randomly divided into four groups, each containing six worms. Group I served as the control (distilled water), Group II received the standard drug albendazole (25 mg/ml), while Groups III and IV were exposed to the plant extract at concentrations of 25 mg/ml and 50 mg/ml, respectively. For each group, 10 ml of the desired formulation solution was poured in separate petri dishes at room temperature, and the worms were introduced into these preparations.

The worms were continuously monitored for their viability. Gentle external stimuli were applied to confirm the motility in live earthworms. The time taken for paralysis were recorded when spontaneous movement ceased, except upon vigorous shaking. The time for death was noted when no movement occurred even after vigorous shaking or immersion in warm water (50 °C), which was accompanied by progressive discoloration of the worm's body [13].

**Statistical analysis**

Data obtained were expressed in mean ± SEM. The level of significance between the groups were analysed by performing One way ANOVA (Analysis of Variance) and subsequent post hoc (Bonferroni test) using IBM SPSS Package. A probability of $P<0.05$ was considered significant.

**Results and Discussion**

**Anthelmintic activity of Hydroethanolic leaf extract of *Hydrocotyle verticillata***

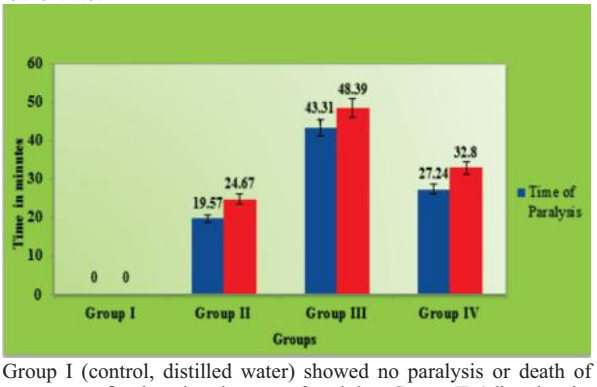
The hydroethanolic leaf extract of *Hydrocotyle verticillata* was evaluated for anthelmintic activity in comparison with albendazole (25 mg/dl). The results are presented in Table 1 and Figure 1.

**Table 1: In vitro anthelmintic activity of Hydrocotyle verticillata Thunb leaf extract**

| Groups | Treatment | Time of paralysis (min) | Time of Death (min) |
|---|---|---|---|
| I | Control – Distilled Water | - | - |
| II | Standard – Albendazole (25 mg/dl) | 19.57 ± 0.51* | 24.67 ± 1.31* |
| III | (Leaf extract at a dose of 25 mg/dl) | 43.31 ± 1.32* | 48.39 ± 1.19* |
| IV | (Leaf extract at a dose of 50 mg/dl) | 27.24 ± 1.27* | 32.80 ± 1.24* |

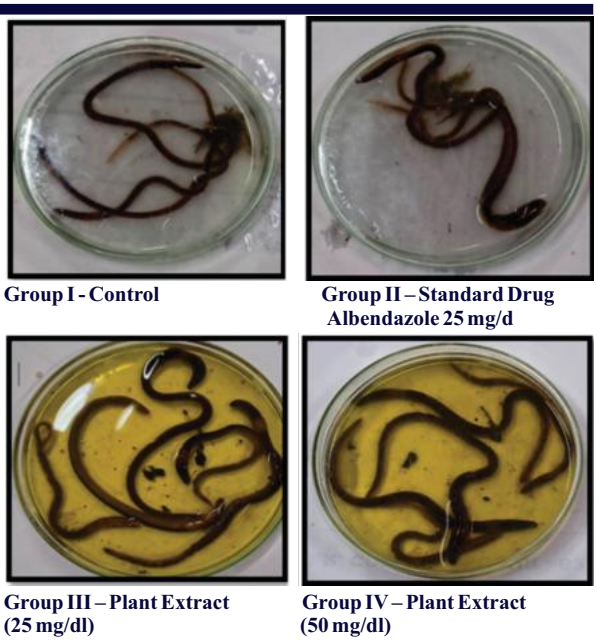
n = 6 in each group, values are mean ± SEM. * were used to compare with groups 2 and 3 respectively, $p< 0.001$

**Figure 1: Anthelmintic activity of Hydrocotyle verticillata Thunb leaf extract**




Group I (control, distilled water) showed no paralysis or death of worms, confirming the absence of activity. Group II (albendazole, 25 mg/dl) exhibited significant efficacy, inducing paralysis at 19.57 minutes and death at 24.67 minutes. At 25 mg/dl (Group III), the extract induced paralysis at 43.31 minutes and death at 48.39 minutes, indicating moderate activity but slower onset compared to albendazole. At 50 mg/dl (Group IV), efficacy improved, with paralysis at 27.24 minutes and death at 32.80 minutes, demonstrating a clear dose-dependent increase in activity (Table 1; Figure 1).

The activity may be attributed to alkaloids, phenolics, terpenoids, and fatty acid derivatives identified in GC–MS analysis. These phytochemicals are known to impair worm motility and survival through disruption of energy metabolism, neuromuscular inhibition, and oxidative stress [14].



Group I - Control | Group II – Standard Drug Albendazole 25 mg/d

Group III – Plant Extract (25 mg/dl) | Group IV – Plant Extract (50 mg/dl)

**Figure 2 : In vitro anthelmintic activity of Hydrocotyle verticillata Thunb leaf extract**

## CONCLUSION

Overall, *Hydrocotyle verticillata* Thunb exhibits measurable anthelmintic potential, though less potent than albendazole. The dose-dependent activity supports its traditional medicinal use and suggests that higher concentrations may enhance efficacy. Further studies are required to isolate the active constituents and confirm the mechanisms of action.

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