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Comparative Evaluation of the *In Vitro* Anthelmintic Activity of Ethanolic Extracts of *Hibiscus hispidissimus* Leaves, *Tamarindus indica* Pulp, and *Carica papaya* Leaves Using *Pheretima posthuma*

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ABSTRACT

Helminthic infections remain a major public health concern, particularly in developing countries, due to poor sanitation and limited healthcare access. Increasing resistance to conventional anthelmintic drugs has encouraged the search for alternative plant-based therapies. The present study aimed to evaluate the *in vitro* anthelmintic activity of ethanolic extracts of *Tamarindus indica* (pulp), *Carica papaya* (leaves), and *Hibiscus hispidissimus* (leaves) using *Pheretima posthuma* as an experimental model. The plant materials were collected, authenticated, shade-dried, and extracted using ethanol. The extracts were evaluated at concentrations of 5, 10, and 20 mg/mL, with albendazole (10 mg/mL) as the standard drug. Anthelmintic activity was assessed by recording the time required for paralysis and death of the worms. Results were expressed as Mean ± SD (n = 6) and analysed using one-way ANOVA followed by Dunnett's multiple comparison test. All three extracts exhibited concentration-dependent anthelmintic activity. Among them, *Hibiscus hispidissimus* demonstrated the highest activity, producing paralysis and death within 3 ± 0.2 min and 6 ± 0.3 min, respectively, at 20 mg/mL. This activity was greater than that observed with albendazole, which produced paralysis and death within 8 ± 0.3 min and 12 ± 0.5 min, respectively. *Carica papaya* exhibited moderate activity, whereas *Tamarindus indica* showed comparatively lower activity. The findings suggest that *Hibiscus hispidissimus* possesses promising *in vitro* anthelmintic potential. Further phytochemical characterization, toxicity studies, and *in vivo* investigations are required to establish its efficacy and safety for potential therapeutic applications.

Keywords: *Hibiscus hispidissimus*, *Carica papaya*, *Tamarindus indica*, Anthelmintic activity, *Pheretima posthuma*.

INTRODUCTION

Helminthiasis is one of the most common parasitic infections affecting humans worldwide, particularly in developing countries with poor sanitation and hygiene. Helminth parasites inhabit the gastrointestinal tract and cause adverse effects such as blood loss, malabsorption of nutrients, intestinal obstruction, anaemia, and the release of toxic metabolites. Anthelmintic drugs are substances that either kill parasitic worms (vermicides) or expel them from the body (vermifuges) [1]. The most common human helminth infections are caused by soil-transmitted helminths (STHs), including Ascaris lumbricoides, Trichuris trichiura, Necator americanus, and Ancylostoma duodenale, followed by schistosomiasis and lymphatic filariasis [2]. These infections contribute significantly to the global burden of Neglected Tropical Diseases (NTDs), and approximately 85% of the NTD burden among the poorest populations in Sub-Saharan Africa is attributed to helminth infections [3]. According to the World Health Organization, more than two billion people worldwide are affected by helminthiasis [4].

Among intestinal helminths, Ascaris lumbricoides infection is often asymptomatic, although heavy worm infestations can result in pulmonary manifestations, intestinal disturbances, and serious complications such as intestinal obstruction [5]. Hookworm infections are among the most pathogenic helminth infestations because the parasites attach to the intestinal mucosa and feed on blood, leading to chronic gastrointestinal blood loss, iron deficiency, and severe anaemia. Enterobius vermicularis (pinworm) infection is highly contagious and commonly spreads through autoinfection. It is characterized by intense perianal itching, which may result in skin irritation, secondary bacterial infections, and sleep disturbances [6]. Chronic helminth infections are associated with malnutrition, impaired growth and cognitive development, reduced productivity, and decreased quality of life, emphasizing the need for effective and affordable anthelmintic therapies.

Medicinal plants have been used since ancient times for the prevention and treatment of various diseases, and their therapeutic applications are well documented in traditional systems of medicine and ancient texts such as the Vedas [7]. India possesses a rich diversity of medicinal plants containing bioactive constituents such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds, many of which exhibit potent pharmacological activities [8]. The increasing prevalence of resistance to conventional anthelmintic drugs has stimulated interest in the search for plant-based alternatives. The plant kingdom is recognized as an important source of natural anthelmintic agents, and several medicinal plants have demonstrated efficacy against parasitic infections in both humans and animals [9].

Among the medicinal plants selected for the present study, *Hibiscus hispidissimus* has been traditionally used in the Kerala region for the management of hepatitis and ocular disorders, while its roots are employed as cooling agents and for the treatment of toxins, swellings, and renal ailments [10][11][12]. *Carica papaya* Linn. (papaya), belonging to the family Caricaceae, is widely recognized for its nutritional and therapeutic value and has been reported to possess anti-inflammatory, antidiabetic, antiviral, anticancer, and anthelmintic activities [13]. *Tamarindus indica* Linn., a member of the family Fabaceae, is an important tropical medicinal plant whose leaves possess anthelmintic and vermifuge properties, while its seeds contain tannins that are reported to be effective against intestinal helminths [14][15]. In addition, various parts of the plant exhibit antioxidant, antidiabetic, anti-inflammatory, and hepatoprotective activities [16][17]. Therefore, these plants were selected for evaluation of their anthelmintic potential using adult Indian earthworms (*Pheretima posthuma*), a well-established experimental model due to its anatomical and physiological resemblance to human intestinal helminths [18].

METHODOLOGY

Collection and authentication of Herbal materials

The leaves *Hibiscus hispidissimus, Tamarindus indica* Pulp, and *Carica papaya* Leaves were collected from the local area and authenticated by Taxonomist.

Preparation of ethanolic extract of Hill hemp bendy, Papaya and Tamarind

The plant materials, *Hibiscus hispidissimus* leaves, *Tamarindus indica* pulp, and *Carica papaya* leaves, were collected from suitable sources and authenticated by a qualified taxonomist. The collected materials were thoroughly washed with water to remove adhering dirt and other contaminants. The plant materials were then be shade-dried, coarsely powdered using a mechanical grinder, and stored in airtight containers until further use. Approximately 50 g of each powdered plant material were packed separately in a Soxhlet apparatus and extracted with 200 mL of ethanol as the extraction solvent. The extraction was carried out at approximately 78°C, corresponding to the boiling point of ethanol, for about 5–6 hours or until the solvent in the siphoning tube becomes colourless, indicating complete extraction of the phytoconstituents. The obtained extracts were filtered through Whatman No. 1 filter paper to remove particulate matter. The filtrates were concentrated under reduced pressure using a rotary vacuum evaporator at 40–45°C to remove the solvent and obtain concentrated extracts. The concentrated extracts were weighed to determine the percentage yield and stored in airtight containers under refrigerated conditions until further use. The prepared ethanolic extracts were subsequently be subjected to preliminary phytochemical screening and evaluation of anthelmintic activity [19][20][21].



Fig 1. Dried Leaves, Soxhletion extraction and Evaporation of papaya



Fig 2. Dried leaves, Soxhlation of extraction and Evaporation of *Hibiscus hispidissimus*



Fig 3. Dried Tamarind pulp, Soxhlation of Tamarind pulp and Evaporation of Tamarind pulp

Preliminary Phytochemical Screening of Plant Extracts

The ethanolic extract of *Hibiscus hispidissimus* (Hill hemp bendy), *Carica Papaya* (papaya) and *Tamarindus indica* (Tamarind) were subjected to preliminary phytochemical screening using standard procedure [22].

Experimental Animal (Worm) Used in the Study

Pheretima posthuma (Indian Earthworm)

Pheretima posthuma is a species of Indian earthworm widely used as an experimental model in anthelmintic studies. It is preferred because its anatomical and physiological characteristics closely resemble those of human intestinal helminths, making it a suitable organism for the preliminary screening of anthelmintic agents. The earthworm is readily available, inexpensive, and can be easily maintained under laboratory conditions, which further supports its extensive use in pharmacological and toxicological research. Owing to its sensitivity to various anthelmintic drugs and plant-derived compounds, *Pheretima posthuma* serves as a reliable and reproducible in vitro model for evaluating the anthelmintic potential of natural and synthetic substances.

In anthelmintic investigations, the efficacy of test compounds is assessed by determining the time required to induce paralysis and death of the worms. A reduction in paralysis and death time indicates greater anthelmintic activity of the test substance. Therefore, *Pheretima posthuma* has become one of the most widely accepted experimental models for the preliminary evaluation of anthelmintic agents [23].

Worm Gathering Process

Indian adult earthworms (*Pheretima posthuma*) were collected from the vermicomposting area of a local plant nursery due to their anatomical and physiological similarities to human intestinal roundworm parasites. The collected earthworms were thoroughly washed with normal saline to remove any adhering soil particles and faecal matter, thereby ensuring their suitability for the anthelmintic study. Only healthy earthworms measuring approximately 3–5cm in length and 0.1–0.2cm in width were selected and used for the experimental procedures [24].

Assessment of Anthelmintic Activity

In vitro anthelmintic activity of the ethanolic extracts of *Hibiscus hispidissimus* leaves, *Tamarindus indica* pulp, and *Carica papaya* leaves were evaluated using adult Indian earthworms (*Pheretima posthuma*). Healthy earthworms measuring approximately 3–5 cm in length and 0.1–0.2 cm in width were selected for the study. Prior to the experiment, the worms were washed thoroughly with normal saline to remove adhering dirt and faecal matter.

The earthworms were divided into five groups, each containing six worms (n = 6):

- Group I (Control): 2% Tween 80 in normal saline.

- Group II (Standard): Albendazole or Piperazine citrate (10 mg/mL).
- Group III (Test I): Plant extract at 5 mg/ml.
- Group IV (Test II): Plant extract at 10 mg/ml.
- Group V (Test III): Plant extract at 20 mg/ml.

Separate sets of groups were maintained for the extracts of *Hibiscus hispidissimus*, *Tamarindus indica*, and *Carica papaya*. All test and standard solutions were freshly prepared before the commencement of the experiment. The extracts were dissolved in a minimum quantity of 2% Tween 80, and the volume were adjusted to 10 mL using normal saline.

The earthworms were placed individually in Petri dishes containing 10 mL of the respective test, standard, or control solution. Anthelmintic activity was assessed by recording the time required for paralysis and death of the worms. Paralysis time were noted when the worms cease movement except upon vigorous shaking, while death time were recorded when the worms fail to respond to external stimuli and exhibit complete loss of motility. The results were expressed as mean ± SEM and compared with the standard drug [25][26].

Statistical Analysis

All data were expressed as Mean±SEM. The statistical significance between groups was compared using one-way ANOVA, followed by Tukey's multiple comparison tests.

RESULTS

Extraction of Plant Material

Crude extracts of *Hibiscus hispidissimus* (Hill hemp bendy), *Carica papaya* (Papaya) and *Tamarindus indica* (Tamarind) were successfully prepared using ethanol as a solvent in this extraction method. The appearance and consistency of each extract are shown below.

Table 1. Characteristics of Herbal Extract

Herbal	Solvent	Color and consistency
Hibiscus hispidissimus (Hill hemp bendy)	Ethanol	Brown
Carica papaya (Papaya)	Ethanol	Dark green
Tamarindus indica (Tamarind)	Ethanol	Brownish-red

Preliminary Phytochemical Screening

Results of the preliminary phytochemical investigation of various herbal extracts is shown in the table.

Table 2. Results of preliminary phytochemical screening

Sl.no	Phytoconstituents	*Hibiscus hispidissimus* (Hill hemp bendy)	*Carica papaya* (Papaya)	*Tamarindus indica* (Tamarind)
1.	Alkaloids	Present	Present	Present
2.	Glycosides	Present	Present	Present
3.	Cardiac glycosides	Present	Present	Present
4.	Flavonoids	Present	Present	Present
5.	Phenolic compounds	Present	Present	Present
6.	Tannins	Present	Present	Present

Anthelmintic Assay



Fig. 4. Control and Standard (Albendazole10mg/ml)



Fig. 5. ***Hibiscus hispidissimus*** **5mg/ml, 10mg/ml and 20mg/ml respectively**



Fig. 6. ***Tamarindus indica*** **5mg/ml, 10mg/ml and 20mg/ml respectively**



Fig. 7. ***Carica papaya*** **5mg/ml, 10mg/ml and 20mg/ml respectively**

Anthelmintic Activity of Selected Medicinal Plants Using *Pheretima posthuma*

Table 3. Anthelmintic Activity of Plant Extracts and Standard Drug

Treatment	Concentration (mg/mL)	Time of Paralysis (min)	Time of Death (min)
Tamarindus indica (Pulp Extract)	5	52 ± 1.5^{ns}	68 ± 2.0^{ns}
	10	38 ± 1.2*	54 ± 1.8*
	20	24 ± 0.8**	39 ± 1.3**
Carica papaya (Leaf Extract)	5	45 ± 1.4*	60 ± 1.7*
	10	31 ± 1.0**	46 ± 1.5**
	20	18 ± 0.6***	32 ± 1.2***
Hibiscus hispidissimus (Leaf Extract)	5	26 ± 0.9**	33 ± 1.1**
	10	11 ± 0.5***	15 ± 0.7***
	20	3 ± 0.2***	6 ± 0.3***
Albendazole (Standard)	10	8 ± 0.3	12 ± 0.5

Values are expressed as Mean ± SD (n = 6). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad Prism software. Differences were considered statistically significant at: ns – Not significant ($P > 0.05$), * – Significant ($P < 0.05$), ** – Very significant ($P < 0.01$), *** – Highly significant ($P < 0.001$), **** – Extremely significant ($P < 0.0001$)
The results were compared with the standard drug, Albendazole (10 mg/mL).

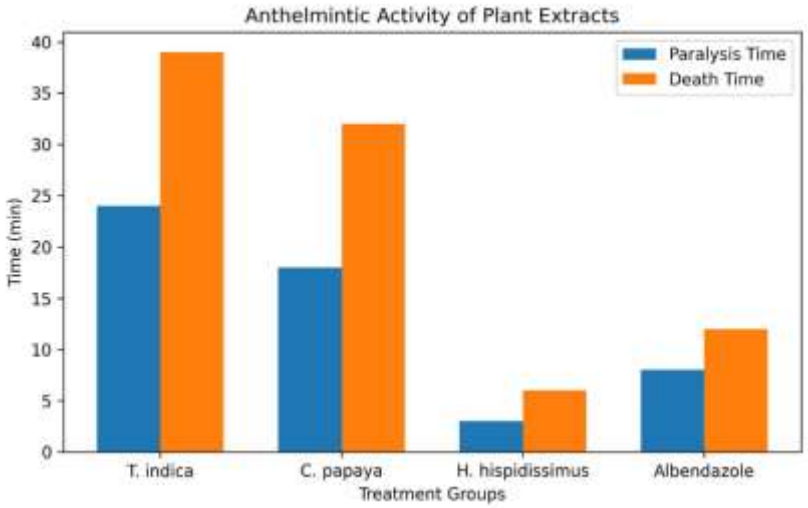

Fig. 8. Graphical representation of the paralysis and death time of earthworms treated with the herbal extract and the standard anthelmintic drug

The anthelmintic activity of ethanolic extracts of *Tamarindus indica*, *Carica papaya*, and *Hibiscus hispidissimus* was evaluated using adult earthworms (*Pheretima posthuma*). The activity was assessed by measuring the time required for paralysis and death of worms at concentrations of 5, 10, and 20 mg/mL. Albendazole (10 mg/mL) was used as the standard reference drug. Among the tested extracts, *Hibiscus hispidissimus* exhibited the most potent anthelmintic activity. At a concentration of 20 mg/mL, the extract induced paralysis within 3 ± 0.2 minutes and death within 6 ± 0.3 minutes. This activity was found to be comparable to that of Albendazole. *Carica papaya* demonstrated moderate activity with paralysis and death times of 18 ± 0.6 and 32 ± 1.2 minutes, respectively, at 20 mg/mL. Conversely, *Tamarindus indica* showed comparatively lower activity, producing paralysis and death at 24 ± 0.8 and 39 ± 1.3 minutes, respectively. A concentration-dependent reduction in paralysis and death times was observed for all plant extracts, indicating increased efficacy with increasing concentration.

DISCUSSION

Helminthic infections continue to pose a significant public health challenge, particularly in developing countries. Medicinal plants have long been recognized as a valuable source of anthelmintic agents due to the presence of bioactive phytoconstituents such as alkaloids, tannins, flavonoids, saponins, and phenolic compounds.

In the present study, the ethanolic extracts of *Tamarindus indica*, *Carica papaya*, and *Hibiscus hispidissimus* were investigated for their anthelmintic potential against *Pheretima posthuma*, which serves as a suitable model because of its physiological and anatomical resemblance to human intestinal parasites. The results demonstrated a clear dose-dependent response for all extracts. As the concentration increased from 5 mg/mL to 20 mg/mL, the time required for paralysis and death of the worms decreased significantly. This observation suggests that the active phytochemical constituents are responsible for the enhanced anthelmintic activity at higher concentrations.

Among the extracts, *Hibiscus hispidissimus* exhibited the highest activity. The remarkable efficacy of this extract may be attributed to the presence of tannins and flavonoids, which are known to interfere with the energy metabolism of helminths and disrupt the integrity of the cuticle, ultimately leading to paralysis and death. Similar findings have been reported for other species of the genus *Hibiscus*, which possess potent antiparasitic and antioxidant properties. *Carica papaya* displayed moderate anthelmintic activity. The observed effects can be attributed to phytoconstituents such as papain, alkaloids, and flavonoids. Papain, a proteolytic enzyme, has been reported to digest the cuticular proteins of helminths, thereby impairing their survival and mobility. *Tamarindus indica* showed comparatively lower activity among the three extracts. Nevertheless, the extract demonstrated a concentration-dependent effect, suggesting the presence of bioactive compounds capable of exerting anthelmintic action, although at a lesser potency.

Albendazole, the standard drug used in the study, exhibited the shortest paralysis and death times among the tested samples. Albendazole exerts its anthelmintic action by inhibiting microtubule polymerization and glucose uptake in helminths, leading to energy depletion and eventual death. The comparison with the standard drug confirmed the significant activity of *Hibiscus hispidissimus* and validated the experimental methodology employed in the study.

Overall, the findings of the present investigation indicate that *Hibiscus hispidissimus* possesses promising anthelmintic properties and may serve as a potential source for the development of novel herbal anthelmintic

formulations. Further studies involving isolation of active constituents, toxicity evaluation, and in vivo pharmacological investigations are warranted to establish its therapeutic potential.

CONCLUSION

The present study was undertaken to evaluate the anthelmintic activity of ethanolic extracts of *Tamarindus indica*, *Carica papaya*, and *Hibiscus hispidissimus* using *Pheretima posthuma* as the experimental model. The findings revealed that all the tested extracts exhibited appreciable anthelmintic activity, as evidenced by their ability to induce paralysis and death of the worms in a concentration-dependent manner.

Among the three plant extracts evaluated, *Hibiscus hispidissimus* demonstrated the most significant anthelmintic activity, producing the shortest paralysis and death times at all tested concentrations. Its activity at 20 mg/mL was found to be comparable to that of the standard drug Albendazole, suggesting the presence of potent bioactive constituents responsible for its pharmacological effect. *Carica papaya* exhibited moderate efficacy, while *Tamarindus indica* showed comparatively lower activity; however, both extracts displayed a clear dose-dependent response, indicating their potential as natural anthelmintic agents.

The observed anthelmintic activity may be attributed to the presence of phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, and proteolytic enzymes. These compounds are known to interfere with the metabolic processes of helminths, damage their cuticular structure, inhibit nutrient uptake, and ultimately cause paralysis followed by death. The results obtained in this study are in agreement with previous reports on the anthelmintic potential of medicinal plants and support the traditional use of these species in the management of parasitic infections.

Furthermore, the study validates the use of *Pheretima posthuma* as a reliable in vitro model for screening anthelmintic agents due to its anatomical and physiological similarities with human intestinal nematodes. The concentration-dependent activity observed across all extracts indicates that increasing the dose enhances the efficacy of the extracts, thereby providing important information for future dosage optimization and formulation development.

In conclusion, the results of the present investigation establish *Hibiscus hispidissimus* as the most promising candidate among the selected medicinal plants for the development of herbal anthelmintic formulations. The study highlights the importance of medicinal plants as a valuable source of novel therapeutic agents that may offer safer, cost-effective, and accessible alternatives to conventional synthetic drugs, particularly in regions where helminthic infections remain prevalent. However, the present work is limited to in vitro evaluation. Therefore, further studies involving isolation and characterization of active phytoconstituents, detailed mechanism of action studies, acute and chronic toxicity assessments, in vivo efficacy studies, and clinical investigations are necessary to substantiate the therapeutic potential of these extracts. Such studies may pave the way for the development of standardized herbal formulations with improved efficacy and safety profiles.

Overall, the study provides scientific evidence supporting the anthelmintic potential of the selected medicinal plants and contributes to the growing body of research on plant-based therapeutics and may be considered for further phytochemical and pharmacological investigations.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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