

EVALUATION OF ANTIHELMINTIC ACTIVITY OF COMBINATION OF CARICA PAPAYA AND TINOSPORA CORDIFOLIA LEAF EXTRACTS USING PHERETIMA POSTHUMA

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ABSTRACT

The present study was carried out to evaluate the anthelmintic activity of aqueous leaf extracts of Carica papaya and Tinospora cordifolia individually and in combination using Pheretima posthuma as an experimental model. The extracts were prepared using the aqueous maceration method. Anthelmintic activity was assessed by recording paralysis time and compared with Albendazole as the standard drug. The results demonstrated that both individual extracts possessed notable anthelmintic activity in a dose-dependent manner, while the combination of extracts exhibited enhanced activity when compared to the individual extracts, particularly showing better efficacy than Tinospora cordifolia alone. The combined extract produced a significant reduction in paralysis and death time, indicating improved wormicidal potential. However, the activity of the herbal combination remained lower than that of standard Albendazole, which showed the highest efficacy. The

enhanced activity of the combination may be attributed to the presence of phytoconstituents such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which may contribute either individually or synergistically to the observed anthelmintic effect. The findings suggest that combining the two plant extracts may produce an additive to mild synergistic interaction, improving their therapeutic potential. In conclusion, the study

supports the traditional use of *Carica papaya* and *Tinospora cordifolia* in helminthic infections and indicates that their combination could serve as a promising natural alternative for anthelmintic therapy, although further studies are required to isolate active constituents, understand the mechanism of action, and validate efficacy through in vivo investigations.

KEYWORDS: Anthelmintic activity, Albendazole, *Pheretima posthuma*, *Carica papaya* and *Tinospora cordifolia*.

1. INTRODUCTION

Helminths is a Greek word meaning "worm". It was supposed to be used only for intestinal worms but now includes tissue parasite free living species and mainly other worms. Helminth infection are common parasitic infection affecting large number of populations, especially children. Helminths could be classified into three classes as Nematoda, Cestoda and Trematoda. People living in Australia, south-east Asia, India, Mexico, Sri Lanka, and Thailand are most affected by this infection. African people living in Sub-Sahara region are one of the most affected peoples. From the current assessment 12-13% of world population have been infected by helminths which causes to severe morbidity it occurs due to persistence shortage, reduction in efficiency and poor socio-economic growth. Inappropriate sanitization is the primary reason of the Helminths infections in peoples. They mainly enter through contaminated drinking water or raw meats from infected animals. They may also get enter in body through skin by insect bites or walking and swimming in contaminated soil and water. Helminth infections can impair cognition in young children through their life cycle, where worms reproduce sexually to produce eggs that continue the infection. Abdominal symptoms from these infections include pain and diarrhea.^[1-3]

ANTHELMINTIC ACTIVITY

Anthelmintic drugs are drugs which are used for treatment of helminthic infections caused by various worms. These are the drugs which act locally to emit the worms from GIT or may also act systematically to eliminate adult helminths and prevent tissue and organs. from developmental forms. Anthelmintic drugs usually kill worms by either starving them to death or paralyzing them as worms don't stores the energy. Anthelmintic drugs show paralytic action due to this they don't have ability to uphold their position in gut. One of the most common synthetic anthelmintic drug used is Albendazole. It acts by blocking the glucose uptake of larvae; thus, it decreases the level of glycogen in adult which leads to decrease in formation of ATP, due to this the worms get immobilized and leads to death. Anthelmintic

drugs effectively treat helminth infections by targeting worms in the gastrointestinal tract or systemically, eliminating adults and developmental stages.

MECHANISM:- These drugs typically paralyze worms by hyperpolarizing nerves or inhibiting motility, preventing attachment to gut walls, or starve them via energy depletion, as worms lack significant reserves.^[4,5]

PAPAYA LEAVES

Papaya leaves are derived from the papaya tree. The botanical name for the papaya plant is *Carica papaya*, which is native to tropical regions. Known for its nutritional benefits, papaya is often regarded as one of the best fruits to consume daily for maintaining good health and fitness. Traditionally, papaya leaves have been utilized for various ailments, including digestive issues, skin problems, and even as a remedy for certain fevers. With the rise of interest in natural remedies and holistic health, papaya leaves are increasingly recognized for their potential benefits. Papaya leaves grow large and are used for their health benefits such as improving digestion, treating infections, and boosting platelet counts especially in dengue fever cases. They exhibit antimicrobial, immune boosting antioxidant, anticancer, and hypoglycemic effects. Key compounds in papaya leaves include carpaine (alkaloid), papain(digestive enzyme) Though generally safe short-term, pregnant women and those with liver impairment should avoid use due to potential toxicities. Papaya leaf extract also helps reduce alcohol-induced stomach damage and modulates immune responses by enhancing certaintcytokines.^[6,7]



Fig. 1: Papaya Leaves.

TINOSPORA LEAVES

Tinospora is also known as “*GILOY, GUDUCHI, AMRUTHAVALLI*”. Guduchi which goes by the scientific name *Tinospora cordifolia* is an extremely essential immune booster herb and a base ingredient of numerous Ayurvedic formulations. Deemed as ‘Queen Of Herbs’, Guduchi plays a significant role in the promotion and restoration of health and curing several diseases and infections. The holistic remedial science of Ayurveda prizes Guduchi as ‘Prana’, i.e., full of vigour as it is very well capable of sustaining and growing on its own even from a small plant part. The renowned Ayurvedic seer Charaka classifies this herb as Medhya Rasayana (a herb which replenishes intellectual capabilities and memory power) and Vayasthapana (herb which arrests ageing). Guduchi is a large, herbaceous, succulent, deciduous, glabrous, climbing shrub that grows about 1.0 meters tall and 0.5 meters wide.^[8,9]

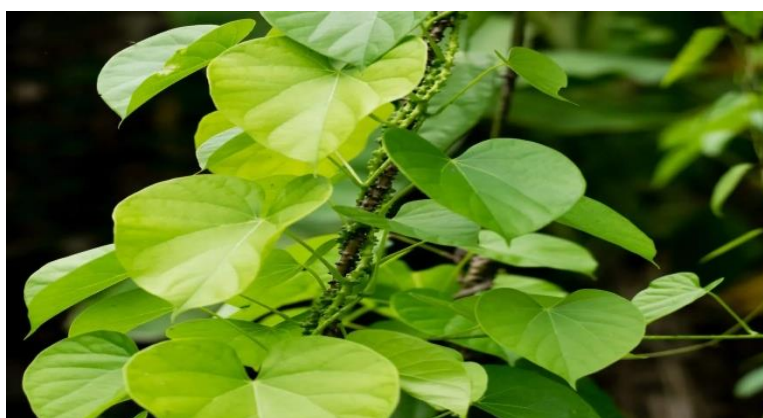


Fig. 2: Tinospora Leaves.

MACERATION

Maceration is a simple, ancient extraction technique widely used in pharmacognosy and phytochemistry for isolating bioactive compounds from plant materials. It involves soaking coarsely powdered or ground plant material in a solvent (such as water, ethanol, methanol, or oils) at room temperature for an extended period, typically days to weeks, allowing the cell walls to soften and release soluble constituents into the solvent. The plant material is immersed in the selected solvent (chosen based on the polarity of target phytoconstituents like alkaloids, flavonoids, or tannins) in a ratio of 1:5 to 1:10w/v). The mixture is occasionally agitated to maintain a concentration gradient and enhance diffusion. Extraction occurs until equilibrium is reached, after which the liquid is filtered or decanted. No heat is applied, making it ideal for thermolabile compounds. Low cost, nothermolabile compounds. Low cost, no specialized equipment, suitable for heat-sensitive materials, and preserves volatile compounds.^[10-13]

PARALYSIS TIME

Paralysis time measures the duration from exposure until worms (*Pheretima posthuma*) lose motility and fail to respond to stimuli like a gentle pinprick or tap, indicating initial anthelmintic effect. Paralysis occurs when the worm shows no spontaneous motility or body movement, even after vigorous shaking, but may still respond faintly to stimuli; it fails to revive in normal saline.^[14,15]

DEATH TIME

Death time in anthelmintic assays is the period from exposure until worms (*Pheretima posthuma*) show no movement, even after immersion in warm saline (52°C) for 1-2 minutes, confirming complete lethality. Death is confirmed when the worm exhibits no movement of any kind—even after dipping in warm water (50°C)—and shows body color fading.^[16,17]

2. MATERIALS USED

Plants used: - Papaya and *Tinospora*

Parts used: - Leaves

Chemicals: - Distilled water, Normal saline, Mayer's reagent, Lead acetate, 10% NaOH, Ferric chloride, DMSO, Picric acid, Iodine

Standard drug: - Albendazole (used for comparison)

Test organism: - Earthworms (*Pheretima posthuma*) – collected from moist soil

Apparatus: - Beakers, Petri dishes, measuring cylinder, filter paper, hot plate, weighing balance

3. METHODOLOGY

PREPARATION OF PAPAYA LEAF EXTRACT BY MACERATION^[18]

Take 5gm of papaya leaf powder and transfer this powder in 50 ml of water in a beaker. Cover the beaker with aluminium foil keep it aside for 3 days with regular stirring. After 3 days keep the solvent on water bath at 40 degree celsius. Leave the solvent until it forms semi solid paste occurs. Dry the paste until it becomes powder in shade dry. The obtained powder was found to be 0.6g

$$\% \text{ Yield} = \frac{\text{wt of dried extract powder obtained}}{\text{wt of initial powder}} \times 100$$

$$= \frac{0.6}{5} \times 100$$

$$= 12\%$$

=12%

PREPARATION OF TINOSPORA LEAF EXTRACT BY MACERATION^[19]

Take 5gm of tinospora leaf powder and transfer this powder in 50 ml of water in a beaker. Cover the beaker with aluminium foil keep it aside for 3 days with regular stirring. After 3 days keep the solvent on water bath at 40 degree celsius. Leave the solvent until it forms semi solid paste occurs. Dry the paste until it becomes powder in shade dry. The obtained powder was found to be 0.8g

$$\% \text{ Yield} = \text{wt of dried extract powder obtained} / \text{wt of initial powder} * 100$$

$$= 0.8/5 * 100$$

$$= 80/5$$

$$= 16\%$$

STANDARD DRUG PREPARATION

For evaluation of anti-helminthic activity, 400 mg of Albendazole tablet of 0.8g is taken. 0.5g of albendazole tablet powder is taken from that tablet and transferred into a clean 50 mL beaker. About 1–2mL of Dimethyl Sulfoxide (DMSO) is added to the beaker containing the drug. The mixture is stirred well for 10–15 minutes using a glass rod or magnetic stirrer to ensure proper dissolution, as Albendazole is more soluble in DMSO. Make up this solution to 10ml with Saline. And finally 25mg/ml concentration was obtained.^[20,21]

PHYTOCHEMICAL INVESTIGATIONS

Table-1- Phytochemical Investigations of Papaya leaves Extract.

TEST	EXPERIMENT	OBSERVATION	RESULT
ALKALOIDS	● <i>PICROLONIC ACID TEST</i>:- Sample+picric acid ● <i>WAGNER'S TEST</i>:- Sample + iodine	Yellow colour appearance Reddish brown colour appearance	Presence of alkaloids
FLAVONOIDS	● <i>ALKALINE REAGENT TEST</i>:- Add NaoH soln to extract ● <i>LEAD ACETATE TEST</i>:- Sample+lead acetate soln	Yellow coloration was observed Yellow precipitate was observed	Presence of flavanoids
PHENOLS	● <i>FERRIC CHLORIDE TEST</i> :- Add 3-5% Fecl3 soln to extract	Deep blue or green colour	Presence of phenols
TERPENOIDS	● <i>SALKOWSKI TEST</i>:- Add chloroform to the extract	Reddish brown coloration at interface	Presence of terpenoids
TANNINS	● <i>FERRIC CHLORIDE TEST</i> :- Add 5% Fecl3 slon to sample	Blue-black was observed	Presence of tannins
SAPONINS	● <i>FORTH TEST</i> :- Shake aqueous extract vigorously	Persistent forth formation (lasting > 10 min)	Presence of saponins

Table 2: Phytochemical Investigations of Tinospora Cordifolia leaf extract.

TEST	EXPERIMENT	OBSERVATION	RESULT
ALKALOIDS	● <i>PICROLONIC ACID TEST</i>:- Sample+picric acid ● <i>WAGNER'S TEST</i>:- Sample + iodine	Yellow colour appearance Reddish brown colour appearance	Presence of alkaloids
FLAVONOIDS	● <i>ALKALINE REAGENT TEST</i>:- Add NaOH soln to extract ● <i>LEAD ACETATE TEST</i>:- Sample+lead acetate soln	Yellow coloration was observed Yellow precipitate was observed	Presence of flavanoids
TERPENOIDS	● <i>SALKOWSKI TEST</i> :- Add chloroform to the extract	Reddish brown coloration at interface	Presence of terpenoids
TANNINS	● <i>FERRIC CHLORIDE TEST</i> :- Add 5% FeCl ₃ soln to sample	Blue-black was observed	Presence of tannins
SAPONINS	● <i>FORTH TEST</i> :- Shake aqueous extract vigorously	Persistent forth formation (lasting > 10 min)	Presence of saponins

PHARMACOLOGICAL INVESTIGATIONS

EXPERIMENTAL DESIGN

GROUP 1	Control(Normal Saline Solution)
GROUP 2	Standard (Albendazole)
GROUP 3	Papaya leaves extract
GROUP 4	Tinospora leaves extract
GROUP 5	Combination (Papaya leaves extract+ Tinospora leaves extract)

CONTROL GROUP PROCEDURE (GROUP 1)

We Took a Petri dish and added 10 mL of Normal Saline which was bought from a local shop. We Introduced four earthworms into the Petri dish containing the normal saline. We observed the activity of the worms and recorded the results.^[23]

STANDARD GROUP PROCEDURE (GROUP 2)

Take a petri dish and add 10ml solution of 25mg/ml concentration of albendazole preparation. Introduce 4 earthworms into the petridish and observe and note paralysis time and death time.^[23]

PAPAYA LEAF EXTRACT PROCEDURE(GROUP 3)

Take a petri dish and add 10ml solution of 25mg/ml concentration of papaya leaf extract preparation. Introduce 4 earthworms into the petridish and observe and note paralysis time and death time.^[24]

TINOSPORA LEAF EXTRACT PROCEDURE(GROUP 4)

Take a petri dish and add 10ml solution of 25mg/ml concentration of tinospora leaf extract preparation. Introduce 4 earthworms into the petridish and observe and note paralysis time

and death time.

COMBINATION OF PAPAYA AND TINOSPORA LEAF EXTRACT PROCEDURE (GROUP 5)

Take a petri dish and add 10ml solution of 25mg/ml concentration of papaya and 25mg/ml concentration of tinospora leaf extract preparation. Introduce 4 earthworms into the petridish and observe and note paralysis time and death time.^[25]



Fig. 3: Estimation of Paralysis time & Death time of Earth Worms.

4. RESULTS

Table 3: Time Taken For Paralysis and Death time of Earth Worms.

Test sample	Concentration (mg/ml)	Time taken for paralysis(min)	Time taken for death(min)
Control(Saline Solution)	—	—	—
Standard (Albendazole)	25mg/ml	2.61 ± 0.44	3.436 ± 0.697
Papaya leaves extract	25mg/ml	7.87 ± 0.63	11.175 ± 0.05
Tinospora leaves extract	25mg/ml	21.24 ± 1.19	26.245 ± 1.564
Combination (Papaya leaves extract+ Tinospora leaves extract)	25mg/ml +25mg/ml	6.97 ± 0.69	13.978 ± 0.415

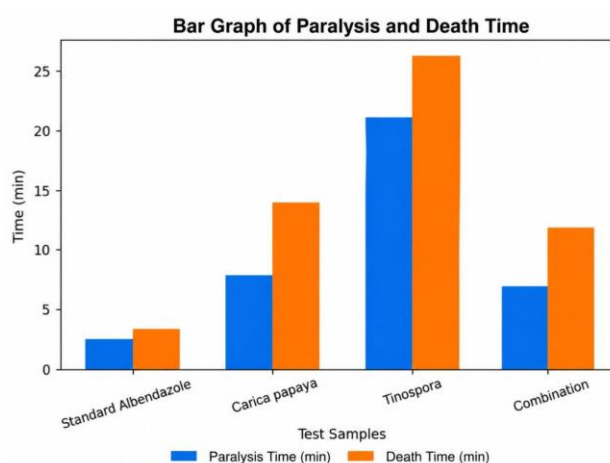


Fig-4- Bar Graph presentation of Paralysis time & Death time of Earth Worms.

5. DISCUSSION

The present study demonstrated promising anthelmintic activity of the aqueous leaf extracts of *Carica papaya* and *Tinospora cordifolia*, both individually and in combination, using *Pheretimaposthuma* as the experimental model. The results were evaluated by recording paralysis time and death time and comparing them with the standard drug Albendazole. Among all the treatments, Albendazole showed the fastest paralysis and death times, confirming its potent and well-established anthelmintic activity. This may be attributed to its known mechanism of action involving inhibition of microtubule synthesis and depletion of energy reserves in helminths, ultimately leading to paralysis and death of worms.

Among the plant extracts tested, *Carica papaya* leaf extract exhibited significant anthelmintic activity. The observed activity may be due to the presence of important phytoconstituents, particularly papain, alkaloids, tannins, flavonoids, and other bioactive compounds. Papain, a proteolytic enzyme, is believed to damage the cuticle of helminths by degrading structural proteins, thereby affecting their survival and motility. Tannins may also contribute by binding to proteins present in the gastrointestinal tract of worms or on the cuticle, leading to death of the parasites. These findings support the traditional use of *Carica papaya* as a natural remedy against helminthic infections.

Tinospora cordifolia leaf extract showed comparatively mild to moderate anthelmintic activity when tested alone. The weaker activity observed may be due to its indirect pharmacological effects, such as immunomodulatory and protective properties, rather than strong direct vermucidal action. Although *Tinospora cordifolia* contains several active constituents such as alkaloids, glycosides, diterpenoids, and phenolic compounds, its effect in this study was lower than that of papaya extract and standard Albendazole.

The combination of *Carica papaya* and *Tinospora cordifolia* extracts demonstrated improved anthelmintic activity compared with *Tinospora cordifolia* alone and showed better efficacy than some individual treatments. This enhanced activity may be attributed to synergistic or additive effects of the combined phytochemicals present in both extracts. The combination possibly improved worm paralysis and death time through multiple mechanisms of action acting simultaneously. Such synergistic interaction supports the concept of polyherbal formulations in enhancing therapeutic efficacy.

However, despite the improved activity shown by the combined extract, its anthelmintic effect did not exceed that of Albendazole. This indicates that while the plant extracts possess

considerable activity, the standard drug remains more potent under the conditions of the present study. Nevertheless, the results suggest that these herbal extracts, especially in combination, may serve as potential natural alternatives or supportive agents for the management of helminthic infections. Further discussion of the results suggests that the dose-dependent response observed in the extracts may be related to the concentration of active phytoconstituents present in the formulations. As the concentration increased, a reduction in paralysis and death time was observed, indicating enhanced anthelmintic efficacy. This trend supports the possibility that higher concentrations of bioactive compounds exert greater toxic or paralytic effects on helminths. Such dose-dependent activity has also been reported in several herbal anthelmintic studies and strengthens the reliability of the present findings.

The use of *Pheretima posthuma* as an experimental model in this study adds relevance to the findings, as its anatomical and physiological similarities to intestinal parasitic worms make it a suitable model for preliminary screening of anthelmintic agents. The paralysis and death of worms observed in response to the extracts indicate direct effects on neuromuscular coordination and structural integrity of the parasites. These effects may arise through disruption of membrane function, interference with energy metabolism, or damage to the protective outer covering of the worms.

Another important aspect of this study is the potential role of phytochemical synergy in the combined extract. Medicinal plants often contain multiple constituents that may act on different targets, producing enhanced overall activity when used together. In the present study, the improved efficacy of the combination may result from the complementary actions of proteolytic enzymes, tannins, alkaloids, and phenolic compounds. This supports the growing interest in polyherbal therapy as a safer and potentially effective alternative to synthetic drugs.

The results of the study also highlight the therapeutic potential of plant-based anthelmintics in addressing issues such as resistance, side effects, and accessibility associated with conventional drugs. With increasing concerns regarding resistance to synthetic anthelmintic agents, medicinal plants may provide a valuable source for discovering novel bioactive compounds. Though Albendazole showed superior activity, the significant effects of *Carica papaya* and the combined extract indicate scope for further development into herbal formulations.

6. CONCLUSION

From the present investigation, it can be concluded that aqueous leaf extract of *Carica papaya* possesses significant anthelmintic activity against *Pheretimaposthuma*, likely due to the presence of papain and other bioactive phytoconstituents. *Tinospora cordifolia* showed mild anthelmintic activity when used alone. The combination of *Carica papaya* and *Tinospora cordifolia* extracts exhibited improved efficacy compared to individual extracts, indicating possible synergistic action between their phytochemicals.

Although the activity of the combined extract was lower than the standard drug Albendazole, the findings support the potential use of these medicinal plants as natural anthelmintic agents. Further studies involving isolation of active constituents, mechanism-based investigations, and *in vivo* evaluations are recommended to validate and enhance their therapeutic potential.

The present study therefore confirms that medicinal plants can serve as valuable sources of bioactive compounds with anthelmintic potential. Among the tested extracts, *Carica papaya* demonstrated comparatively stronger activity, suggesting its possible usefulness as a natural therapeutic agent against helminthic infections. The moderate activity shown by *Tinospora cordifolia* and the improved response observed in the combined extract further emphasize the significance of polyherbal approaches in enhancing biological activity.

The findings also indicate that the observed anthelmintic effect may be concentration dependent, with higher extract concentrations producing better paralysis and death times. This supports the importance of dose optimization in herbal formulations to achieve maximum therapeutic efficacy. The synergistic interaction of phytoconstituents such as papain, tannins, alkaloids, flavonoids, and phenolic compounds may contribute to the improved activity observed in the combined extract.

Although the herbal extracts did not surpass the efficacy of Albendazole, they demonstrated considerable activity and may offer safer, affordable, and accessible alternatives, particularly in areas where synthetic drugs are limited or resistance to conventional anthelmintics is a concern. These results support the traditional medicinal use of these plants and provide a scientific basis for their further exploration.

In conclusion, *Carica papaya* alone and in combination with *Tinospora cordifolia* possesses promising anthelmintic properties and may be considered potential candidates for development of herbal anthelmintic formulations. However, further detailed studies including

phytochemical standardization, toxicity profiling, in vivo experimental models, and clinical evaluation are necessary before their therapeutic application can be fully established.

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