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Phytochemical Profiling and in Vitro Anthelmintic Activity of Nigerian Carica papaya Leaf Extract Against Adult Haemonchus contortus Isolated from Sheep in Northern Cyprus

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Abstract- ***The study aimed to comprehensively evaluate the therapeutic efficacy of both methanolic and aqueous extracts of the leaves of Carica papaya in combating Haemonchus contortus, which serves as a well-established experimental model for helminthic infections. The purpose was to determine whether these plant extracts could offer a viable alternative or complement to conventional anthelminthic treatments. A total of thirty-six adult earthworms, measuring 3.5–5.0 cm in length, were collected from across sheep farms in Northern Cyprus. These worms were randomly allocated into nine experimental groups, with each group containing four worms. Fresh solutions were prepared immediately prior to the experiment, including 0.9% (w/v) normal saline as a control, and 25, 50, and 100 mg/mL concentrations of albendazole (control drug), as well as the ethanolic and aqueous bark extracts of Carica papaya leaves. Each group of worms was exposed to these solutions, and the time taken for paralysis and subsequent death was meticulously recorded in minutes. The methanolic leaf extract demonstrated a clear dose-dependent effect, with increasing concentrations (25, 50, and 100 mg/mL) producing progressively faster paralysis of the earthworms. Data were analyzed using One-way ANOVA and expressed as mean ± SEM. Differences achieving P<0.01 were considered statistically significant, confirming the potency of the methanolic extract compared to the aqueous extract. The findings indicate that the methanolic extract of Carica papaya leaves exhibits superior anthelminthic activity compared to its aqueous counterpart. Moreover, when compared with the control drug albendazole, the methanolic extract demonstrated significant efficacy in immobilizing and killing Haemonchus contortus. These results support the potential use of Carica papaya leaves, particularly its methanolic extract, as a natural therapeutic agent for treating intestinal worm infections, highlighting its promise for further pharmacological development.***

Keywords: Albendazole, Carica papaya, Haemonchus Contortus, Petri dish, Phytochemical

I. INTRODUCTION

Resistance to anthelmintic drugs has become a global issue, prompting research into plant-based compounds as alternative treatments for gastrointestinal nematodes. Helminth infections are often chronic and may contribute more to human and animal morbidity, as well as economic and social hardships, than any other group of parasites (Partap et al, 2012). Helminthiasis affects a significant portion of the global population, involving infestations by worms such as pinworms, roundworms, or tapeworms. While these parasites are usually found in the gastrointestinal system, they can also invade organs like the liver (Raju & Yesuf, 2010). Parasitic worms are categorized into three main types: cestodes (tapeworms), nematodes (roundworms), and trematodes (flukes) (Mali & Mehta, 2008). Infections caused by these parasites can lead to severe diseases, including lymphatic filariasis (which can cause elephantiasis), onchocerciasis (river blindness), and schistosomiasis (Pandey et al., 2011). In many developing countries, poor sanitation, malnutrition, and poverty increase the risk of worm infestations (Tripathi, 2008). Helminth infections can result in

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nutritional deficiencies, anemia, and compromised immune function (Gaikwad et al., 2011). Moreover, most currently available synthetic anti-helminthic drugs have side effects and are often not cost-effective.

Populations that live in close quarters for extended periods such as in schools, orphanages, or densely populated urban slums face a higher risk of helminth transmission and environmental contamination. Helminths are recognized as "masterful immunoregulators" (Borah et al., 2013), capable of triggering complex Th1/Th2 immune responses that simultaneously defend against and manipulate the host's immunity, allowing the parasites to persist for months or even years. Evidence of helminth infections dates back to ancient human civilizations, including findings in mummified feces, references by Hippocrates, and the Ebers Papyrus, a medical text from around 1500 BC (Das et al., 2011, Kumar et al., 2010, Baruah & Nath, 2001, Panda et al., 2011). Despite this long history, our understanding of how these parasites modulate host–parasite interactions and influence human immune responses has only recently begun to expand and remains incomplete (Sampat et al., 2009). This gap in knowledge is partly due to the successful control of these parasites in wealthier regions such as Europe and the USA during the sanitation improvements of the late 19th century (Paria et al., 2012). Consequently, the focus of mid-20th-century biomedical advances, including vaccines and therapies, largely overlooked helminths, and only a few research laboratories continued studying them. For much of this time, these infections continued to disproportionately affect the world's poorest populations. Nevertheless, recent increases in donor funding and government initiatives aimed at improving global public health provide a promising outlook. It is plausible that similar patterns such as high baseline prevalence of Eimeria, extended gastrointestinal nematode (GIN) transmission periods, and potentially undetected resistance pressures occur locally, considering the geographic, climatic, and production characteristics of Northern Cyprus. These include its semi-Mediterranean climate, diverse farming systems, and mixed-age and sex flock management. Nevertheless, region-specific data remain scarce, and in the absence of formal epidemiological studies, any management guidelines remain largely speculative (Olaifa et al., 2025).

Albendazole, a widely used broad-spectrum anthelmintic, has been reported to cause side effects such as nausea, vomiting, dizziness, and irritation of the gastrointestinal tract in some individuals (Borah et al., 2013). Consequently, there is growing interest in the use of herbal remedies as alternative anthelmintic agents. Plant-based treatments are generally more affordable and tend to produce fewer adverse effects compared to synthetic drugs. The anthelmintic potential of such remedies is often tested using adult earthworms, Haemonchus contortus, due to their anatomical and physiological similarity to human intestinal roundworms, as well as their ready availability (Das et al., 2011, Kumar et al., 2010).

Carica papaya Linn, commonly called papaya or pawpaw, is a significant member of the Caricaceae family and is cultivated in tropical and subtropical regions around the world for its nutritional, medicinal, and industrial uses. Numerous studies have explored the biological activities of various parts of the plant, including its fruits, leaves, shoots, seeds, roots, peel, and latex. The leaves of papaya are particularly rich in phytochemicals such as flavonoids, phenolics, and alkaloids (Endres et al., 2025). Both in vitro and in vivo research has shown that papaya leaves exhibit multiple therapeutic effects, including antioxidant, anti-inflammatory, anticancer, antimicrobial, and hepatoprotective properties. Traditionally, papaya leaves have been used to manage parasitic infections, gastrointestinal problems, fever, burns, and asthma, highlighting their value as a source of bioactive compounds beneficial to human health (Airaodion et al., 2020). Key bioactive constituents identified in papaya leaves include papain, flavonoids, alkaloids, tannins, terpenoids, saponins, and polyphenols (Jaji et al., 2020) Among the phenolic compounds reported, phenolic acids such as p-coumaric, caffeic, protocatechuic, and gallic acids are commonly found, alongside flavonoids including kaempferol, quercetin, rutin, and naringenin. Phenolic acids are typically present in higher concentrations, with p-coumaric and caffeic acids being the most abundant, whereas rutin and naringenin dominate among the flavonoids (Masfufatun et al., 2019).

II. MATERIAL AND METHODS

Plant material

Mature leaves of Carica papaya were sourced from the botanical garden of the Department of Botany at Imo State University, Owerri, Imo State, Nigeria. The plant specimens were carefully examined, identified, and formally authenticated by the Chief Botanist of the department to ensure accurate species verification. After collection, the leaves were spread out in a well-ventilated shaded area and allowed to air-dry naturally for a period of ten days to preserve their bioactive compounds. Once dried, the leaves were roughly ground into coarse powder using a clean mortar and pestle and subsequently stored in airtight containers to protect them from moisture, humidity, and direct sunlight. These prepared leaf samples were then transported under controlled conditions to Northern Cyprus for further experimental investigations and in vitro analyses.

Preparation of ethanolic and aqueous extract

A total of approximately 200 grams of coarsely powdered Carica papaya leaves was subjected to extraction using the Soxhlet apparatus. Prior to the main extraction, the powdered material underwent defatting with petroleum ether to remove non-polar impurities and lipids. The extraction was then carried out with 1000 mL of methanol for a continuous period of 18 hours to ensure thorough solubilization of the bioactive constituents. Following extraction, the methanolic solvent was removed under reduced pressure using a rotary evaporator, and the concentrated extract was stored in a desiccator to prevent moisture absorption until further experimental use.

In parallel, an aqueous extract was prepared through cold maceration, allowing water-soluble phytochemicals to diffuse into the solvent at room temperature over a designated period. Preliminary phytochemical screening of the extracts revealed distinct compositions: the methanolic extract was found to contain carbohydrates, glycosides, lignin, saponins, tannins, and phenolic compounds, while the aqueous extract was rich in glycosides, saponins, tannins, and phenolic constituents. These findings highlight the solvent-dependent nature of phytochemical extraction, with methanol preferentially isolating both polar and moderately non-polar compounds, whereas water primarily extracted highly polar bioactive components.

Northern Cyprus adult Haemonchus contortus as model for the experiment

All experimental procedures were conducted using adult Haemonchus contortus collected from the moist soils in a sheep farm across Northern Cyprus. The worms were thoroughly washed with normal saline to remove adhering soil and fecal matter before use. These worms were selected for the anthelmintic activity study because their anatomical and physiological characteristics closely resemble those of the human intestinal roundworm Ascaris lumbricoides, making them a suitable in vitro model for preliminary evaluation of anthelmintic agents. The use of Haemonchus contortus is particularly advantageous due to their ready availability, ease of handling, and ethical feasibility compared to direct human or animal parasite studies. Adult worms used in the experiments measured approximately 3.5–5.0 cm in length and 0.2–0.5 cm in width, providing a standardized size range to ensure consistent results across replicates. Their physiological response to the extracts allows reliable assessment of potential anthelmintic efficacy before advancing to more complex parasitic models.

Drugs and Chemicals

The reference standard drug, albendazole was formulated in distilled water at three varying concentrations: 25 mg/mL, 50 mg/mL, and 100 mg/mL. In a similar manner, both the methanolic extract of Carica papaya leaves and the aqueous extract of Carica papaya leaves were prepared at identical concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL using distilled water as the solvent. These preparations were employed as the test solutions to evaluate their anthelmintic activity under controlled experimental conditions.

Evaluation of anthelmintic activity

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The anthelmintic potential of the methanolic and aqueous extracts of Carica papaya was assessed following the protocol described by Panda et al., 2011). A total of thirty-six adult worms were collected and evenly distributed into nine groups, with four worms assigned to each group. For the experiment, 10 mL of each concentration of the standard drug albendazole and the test extracts were measured using a pipette and placed into nine separate clean Petri dishes. Each group of four earthworms was then introduced into its respective dish. The worms were closely monitored, and the time required for paralysis and death was carefully recorded in minutes. Paralysis was determined when the worms exhibited no movement or response in normal saline, even upon vigorous shaking, indicating complete loss of neuromuscular activity. Death was confirmed when the worms showed total immobility, did not respond to being immersed in warm water (50–60 °C), and displayed a faded body color, indicating complete cessation of physiological activity. This approach allowed a quantitative assessment of the efficacy of the extracts in comparison to the standard anthelmintic drug.

Figure 1. Effect of control drug albendazole on paralysis and death.

Figure 2. Effect of ethanolic extract of Carica papaya on paralysis and death

Statistical analysis

The experimental findings were presented as the mean ± standard error of the mean (SEM), calculated from four earthworms in each treatment group. Statistical analysis was performed to compare the effects observed in the extract-treated groups with those of the standard reference group. These comparisons were conducted using Dunnett's post hoc test, which is specifically designed to evaluate multiple treatment groups against a single control. A probability value (P-value) of less than 0.01 was regarded as statistically significant, indicating that there is less than a 1% likelihood that the observed differences occurred by chance. This threshold reflects a high level of confidence in the reliability and validity of the experimental outcomes.

III. RESULTS AND DISCUSSION

Preliminary phytochemical screening of Carica papaya extract

This screening was performed for confirmation of various types of bioactive compounds that are present in the Carica papaya extract. The phytochemical assessments of the leaf extracts were analyzed and the results were shown in Table 1.

Table 1. Phytochemical screening of *Carica papaya* leaves extract.

No.	Phytochemicals	Test	Remarks	Results
1	Phenols	Ferric chloride test	Dark green	(+)

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2	Saponins	Frothing (foam) test	Foam seen	(+)
3	Flavonoids	Alkaline reagent test	Yellow	(+)
4	Tannins	Lead acetate test	Yellowish precipitate	(+)
5	Alkaloids	Mayer's Test	Reddish-brown precipitate	(+)
6	Glycosides	Keller-Killiani Test	Reddish-brown	(+)
7	Terpenoids	Salkowski Test	Reddish-brown coloration	(+)

(+): indicates the presence of phytochemicals in the Carica papaya leaves extract.

The brownish-green extract obtained from Carica papaya leaves has undergone phytochemical screening tests, which demonstrate the existence of saponins, flavonoids, phenols and tannins.

The findings of the anthelmintic assessment are summarized in Table 2. The findings demonstrate that increasing the concentration of the extracts led to a quicker onset of paralysis and a reduced time to death in the tested earthworms. This confirms a clear dose-dependent response, where higher concentrations produced more pronounced anthelmintic effects. Both the crude methanolic and aqueous extracts, tested at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL, exhibited progressive paralytic activity as the dose increased. The worms became immobilized more rapidly and died sooner at higher concentrations, indicating enhanced potency with increasing extract strength.

At 100 mg/mL, the ethanolic leaf extract of Carica papaya produced paralysis and death at 53.84 ± 0.36 minutes and 70.62 ± 0.09 minutes, respectively ($P < 0.01$). In contrast, the aqueous leaf extract at the same concentration caused paralysis and death at 68.91 ± 0.48 minutes and 72.83 ± 0.37 minutes, respectively ($P < 0.01$). These slightly improved values suggest that the methanolic extract maintained greater efficacy compared to the aqueous preparation, as reflected by the shorter paralysis and mortality times. When compared with the control drug at 100 mg/mL, which induced paralysis and death at 33.76 ± 0.29 minutes and 49.95 ± 0.14 minutes, respectively, both plant extracts showed lower activity. However, the differences were statistically significant ($P < 0.01$), indicating that the extracts possess genuine anthelmintic potential. Overall, the data reinforce the dose-related effectiveness of the extracts, with the methanolic fraction demonstrating comparatively stronger activity. This finding is similar to what Sarmah et al., 2017 demonstrated in table 2.

Table 2: Anthelmintic Activity of Extracts

Drug Treatments	Doses (mg/mL)	Time Taken for Paralysis (min)	Time Taken for Death (min)
Control Drug (Albendazole)	25	44.92 ± 0.42	60.84 ± 0.39
	50	39.76 ± 0.45	58.97 ± 0.41
	100	33.78 ± 0.29	49.95 ± 0.14
MECL	25	66.37 ± 0.28	78.54 ± 0.35
	50	60.84 ± 0.12	74.93 ± 0.44
	100	53.84 ± 0.36	70.62 ± 0.09
AECL	25	79.74 ± 0.50	85.91 ± 2.18
	50	74.62 ± 0.58	81.94 ± 0.55
	100	68.91 ± 0.48	72.83 ± 0.37

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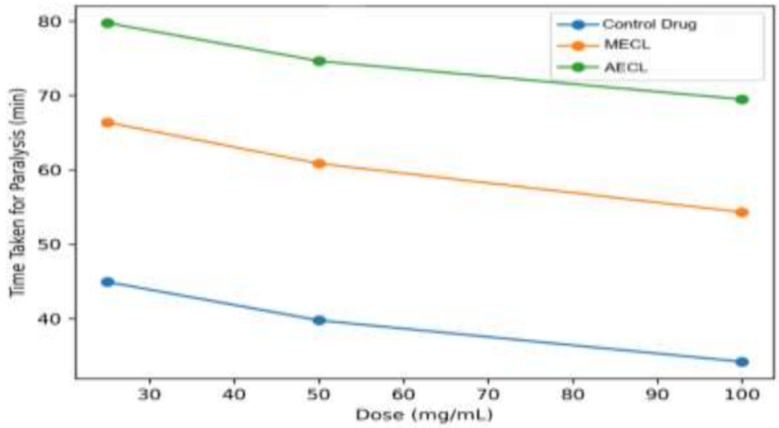
Statistical analysis was carried out with comparisons between control and treated samples. n=4 was taken in each group. Symbols represent statistical significance *P<0.05, **P<0.01.



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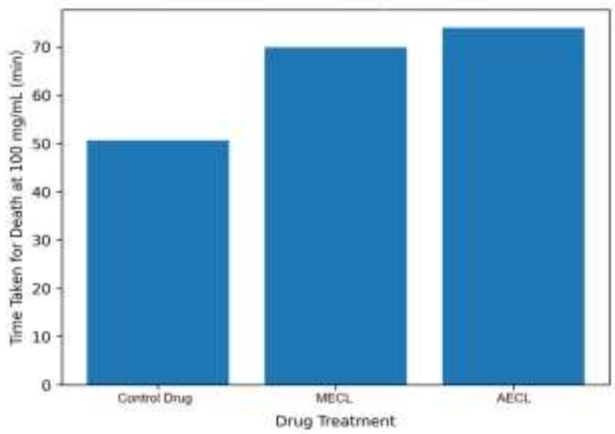

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Figure 3a shows anthelmintic activity of dose vs paralysis time, 3b shows comparison of death time at 100 mg/mL

IV. CONCLUSION

In this study, all experiments were conducted in vitro using the Norther Cyprus adult Haemonchus contortus due to its anatomical and physiological similarity to the intestinal roundworm parasites that infect humans. These parasitic helminths are responsible for persistent infections in both humans and animals. While numerous synthetic drugs and their derivatives have been developed to combat these parasites, their use often comes with significant side effects. Moreover, the emergence of drug resistance in helminths can worsen infections, highlighting the limitations of conventional therapies. As a result, attention has increasingly shifted toward herbal remedies, which are considered safer alternatives for treating helminth infections. The findings of the present study demonstrate promising anthelmintic potential of the plant under investigation, suggesting that it could serve as a natural means to control gastrointestinal nematode infections in humans. Comparative results indicated that the time required for albendazole to induce paralysis and death in the worms was comparable to that observed with the methanol extract of C. papaya leaves (MECL). Preliminary phytochemical screening revealed that MECL contains alkanoids, glycosides, terpenoids, saponins, tannins, and phenolic compounds. Certain phytoconstituents, particularly tannins and phenolics, may contribute

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significantly to its anthelmintic effects. Previous studies have suggested that tannins and phenolic compounds can disrupt energy metabolism in helminths by uncoupling oxidative phosphorylation or by binding to free proteins in the worm's gastrointestinal tract, ultimately leading to parasite death. Additionally, flavonoids and other polyphenolic compounds present in the extract may also play a role in its anthelmintic activity. Further in vivo studies are required to confirm the pharmacological efficacy and safety of Carica papaya as a potential anthelmintic agent for clinical use.

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