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In vitro comparative evaluation of the anthelmintic effects of ethanolic extracts from *Carica papaya* leaves and seeds against *Ascaridia galli*

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Abstract

Gastrointestinal nematodes, particularly *Ascaridia galli*, significantly threaten free-range chicken production, reducing growth and productivity. Raisers commonly use synthetic anthelmintics like levamisole; however, concerns over resistance and cost have driven interest in plant-based alternatives. This study evaluates the in vitro anthelmintic efficacy of ethanolic extracts from *Carica papaya* seeds and leaves against *A. galli*. Freshly collected nematodes from slaughtered native chickens were exposed to seven treatments: T0 (positive control—levamisole), T1–T3 (1 mg/ml, 2.5 mg/ml, and 5 mg/ml ethanolic seed extracts), and T4–T6 (1 mg/ml, 2.5 mg/ml, and 5 mg/ml ethanolic leaf extracts). Worm mortality was assessed at 1, 3, and 6 hours post-exposure. T1 (1 mg/ml seed extract), T3 (5 mg/ml seed extract), and T6 (5 mg/ml leaf extract) exhibited high mortality comparable to levamisole at 6 hours, suggesting strong anthelmintic potential. Phytochemical analysis revealed that tannins, saponins, alkaloids, flavonoids, and glycosides were absent in PLEE, while only alkaloids and flavonoids were detected in PSEE. The absence of these compounds may be due to variations in extraction methods and solvent use rather than their complete absence. Statistical analysis showed significant differences ($p < 0.05$) across treatments and time intervals. These findings suggest that papaya extracts hold promise as natural anthelmintics for poultry parasite control. In vivo research is recommended to ensure safety and practical application in chickens.

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Introduction

Gastrointestinal nematode infections pose significant challenges to chicken production's health and economic stability worldwide, particularly affecting free-ranged chickens, which are highly susceptible to parasitic infestations. Native chicken farming in the Philippines is essential to millions of people's livelihoods and food security (Dusaran, 2015). As of September 2023, the native chicken population in the country is estimated at 80.7 million birds, comprising 43.0% of the total chicken inventory. Western Visayas (Region VI) leads in production with 12.71 million native chickens, followed by Northern Mindanao (Region X) with 12.03 million (PSA, 2023). Despite its increasing economic significance, native chicken production—practiced mainly in backyard and semi-commercial settings—faces persistent threats from gastrointestinal parasitism, which limits productivity.

Traditional anthelmintics like levamisole, ivermectin, and fenbendazole are frequently utilized to treat these parasitic infections. However, parasite resistance has risen due to these medications being administered frequently and incorrectly (Jamil *et al.*, 2022). Alternative veterinary approaches are being investigated in response, emphasizing natural anthelmintics from plant materials such as leaves and seeds. Among these, *Carica papaya*, a member of the Caricaceae family, has shown promise due to its bioactive compounds. Alkaloids, glycosides, tannins, saponins, and flavonoids in papaya leaves (Singh *et al.*, 2020) contribute to their medicinal properties, particularly in combating gastrointestinal nematodes.

Formulating ethnobotanicals as an alternative to synthetic anthelmintics presents a cost-effective and sustainable approach to parasite control in poultry. This strategy mitigates the rising issue of anthelmintic resistance and offers a potential solution to the high costs and limited accessibility of commercial dewormers (Zirintunda *et al.*, 2022). Thus, this study aims to evaluate the anthelmintic efficacy of *Carica papaya* leaves and seeds through ethanolic extraction in alleviating gastrointestinal nematodes in naturally infected chickens. The results of this study will add to the expanding corpus of knowledge on plant-based anthelmintics and their possible uses in animal production and veterinary medicine.

Materials and methods

Materials

This study used leaves and seeds of *C. papaya*, ethanol (95%), an empty container for maceration, a blender, a rotary evaporator for concentration, Petri dishes, a mortar and pestle, NSS, a micropipette, an incubator, and levamisole.

Plant collection and verification

Fresh *C. papaya* leaves and unripe black seeds were collected and taxonomically identified by Prof. Hemres Alburo at the Biodiversity, Environment, and Natural Resources Research Center, Cebu Technological University – Argao Campus.

Extraction procedure

Fresh seeds and leaves were collected, washed thoroughly, and air-dried at room temperature for 15 days, per Peter *et al.* (2014). The dried materials were pulverized, stored in airtight containers, and extracted via cold maceration: 500g of powdered *C. papaya* seeds and leaves were each soaked in 2,800ml of 95% ethanol for 72 hours at room temperature following a modified method by Owoyele *et al.* (2008) and Masfufatun *et al.* (2018). With a circulating water vacuum pump SHZ-DIII (Biobase, China), the extracts were concentrated using a Rotary Evaporator RE-100 Pro (Biobase, China) set to 40°C and 104 rpm. The concentrated extracts were then filtered through Whatman No. 1 filter paper, labeled, and stored at -20°C for subsequent analysis.

Phytochemical screening

The ethanolic extract samples were submitted to the Applied Microbiology Laboratory at the Department of Biology, University of San Carlos-Talamban Campus, for phytochemical screening of tannins, saponins, flavonoids, alkaloids, and glycosides using various test methods.

Collection of Ascaridia galli

Live nematodes were collected from the intestines of freshly slaughtered native chickens at a private slaughter farm in Dumanjug, Cebu. Their viability and motility were confirmed following the method of Amelia *et al.* (2017), after which they were washed, suspended in NSS (0.9% sodium chloride), and transported to the laboratory. The nematodes were counted, divided into seven groups, and incubated at 37°C (Aziz *et al.*, 2018). Worms were assessed for paralysis and mortality by gently probing them with forceps; those exhibiting no movement were classified as immobile or dead. The in vitro efficacy of ethanolic extracts was determined based on the mortality percentage of immobile worms after exposure, as described by Radwan *et al.* (2012). The test extract was placed in 100 mm Petri dishes, with ten worms assigned to each dish.

Mortality (%) = (Number of dead worms)/(Total number of worms) × 100

The experiment consists of seven treatments: T0 (positive control), where worms are exposed to levamisole, and six test treatments: T1, T2, and T3, where worms are exposed to 1 mg/ml, 2.5 mg/ml, and 5 mg/ml of *Carica papaya* seed extract, respectively; and T4, T5, and T6, where worms are exposed to 1 mg/ml, 2.5 mg/ml, and 5 mg/ml of *C. papaya* leaf extract, respectively.

Each treatment was replicated three times to assess anthelmintic effects based on worm motility inhibition. Paralysis or inactivity was recorded at 1, 3, and 6 hours. After six hours, extracts were washed off, and worms were re-suspended in warm NSS (0.9% sodium chloride) for 30 minutes to test motility revival.

Parasite morphology verification

The nematodes were identified at the College of Veterinary Medicine, Visayas State University (ViSCA), by Dr. Harvie P. Portugaliza using stereomicroscopy. Three adult female nematodes, measuring 85 mm, 94 mm, and 88 mm, were collected from a native chicken. The specimens had elongated, cylindrical, yellowish-white bodies covered by a cuticle, tapering at the anterior end with a mouth surrounded by three lips: one dorsal and two subventral. All had blunt, rounded tails.

Experimental design

A Two-Factorial Completely Randomized Design (CRD) was used to evaluate the effects of 7 treatments (Factor A) and three-time intervals (Factor B: 1 hr, 3 hrs, 6 hrs) on worms. Each Petri dish, containing 10 worms, served as a replicate for each treatment-time combination.

Data were collected at each time point, and Two-Way ANOVA was performed to analyze the main effects of treatments and time intervals and their interaction. Experimental flowchart illustrating the step-by-step process of the study, from sample collection to termination of the study is given below (Fig. 1).

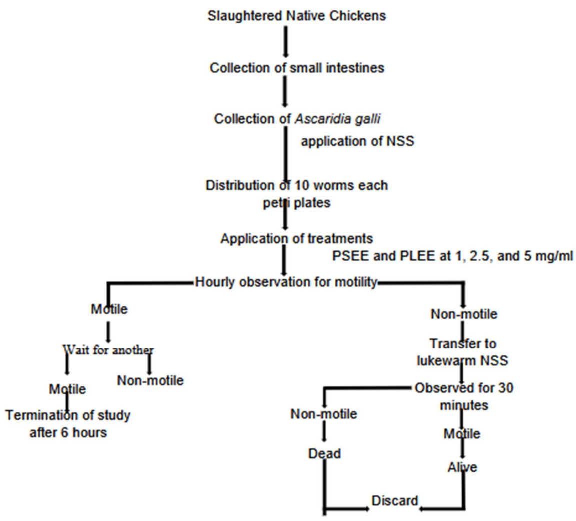

Fig. 1. Experimental flowchart illustrating the step-by-step process of the study, from sample collection to termination of the study

Results and discussion

Please see Table 1 and Table 2 for the summary of mortality percentage of worms and phytochemical screening results of the extracts.

Mean percentage of mortality of Ascaridia galli

Table 1 shows that the mortality of *A. galli* varied across different concentrations of *C. papaya* seed and leaf extracts over time. Treatment 0 (Levamisole) exhibited the highest mortality, achieving 100% at 1 hour and slightly decreasing to 98.89% at 3 hours and 91.11% at 6 hours, reinforcing its potency as a standard anthelmintic. Among the seed extracts, Treatment 1 (PSEE 1 mg/ml) demonstrated a steady increase in mortality, reaching 97.78% at 6 hours, resembling levamisole. In contrast, Treatment 2 (PSEE 2.5 mg/ml) exhibited decisive initial mortality (98.89% at 1 hour) but declined significantly at 3 hours (84.44%), indicating a short-lived effect. Treatment 3 (PSEE 5 mg/ml) fluctuated, with high mortality at 1 hour (96.67%), a decrease at 3 hours (87.78%), and recovery at 6 hours (95.56%), suggesting prolonged but variable efficacy.

Table 1. Mean percentage (%) of mortality of *Ascaridia galli* at varying *Carica papaya* extract concentrations across different observation periods

Treatments	Observation period		
	1 hour	3 hours	6 hours
T0	100.00 [a]	98.89 [a]	91.11 [a]
T1	93.33 [abc]	83.33 [b]	97.78 [a]
T2	98.89 [ab]	84.44 [b]	88.89 [a]
T3	96.67 [abc]	87.78 [ab]	95.56 [a]
T4	87.78 [bc]	88.89 [ab]	92.22 [a]
T5	88.89 [abc]	92.22 [ab]	90.00 [a]
T6	86.67 [c]	84.44 [b]	95.56 [a]
A × B	**		
CV (%)	5.09		

T0 - worms were exposed to Levamisole

T1 - worms were exposed to 1 mg/ml *C. papaya* seed extract

T2 - worms were exposed to 2.5 mg/ml *C. papaya* seed extract

T3 - worms were exposed to 5 mg/ml *C. papaya* seed extract

T4 - worms were exposed to 1 mg/ml *C. papaya* leaf extract

T5 - worms were exposed to 2.5 mg/ml *C. papaya* leaf extract

T6 - worms were exposed to 5 mg/ml *C. papaya* leaf extract

For leaf extracts, Treatment 4 (PLEE 1 mg/ml) and Treatment 5 (PLEE 2.5 mg/ml) maintained relatively stable mortality across time points, with Treatment 5 peaking at 92.22% at 3 hours before slightly decreasing at 6 hours. Treatment 6 (PLEE 5 mg/ml) exhibited the lowest mortality at 1 hour (86.67%) but significantly increased to 95.56% at 6 hours, indicating a dose-dependent and time-dependent relationship.

The results suggest that *C. papaya* extracts exhibit significant anthelmintic properties, with Treatment 1 (PSEE 1 mg/ml), Treatment 3 (PSEE 5 mg/ml), and Treatment 6 (PLEE 5 mg/ml) demonstrating high mortality comparable to levamisole at 6 hours. Bioactive substances such as alkaloids, flavonoids, tannins, and proteolytic enzymes like papain are responsible for this action. These substances interfere with nematode neuromuscular function, causing paralysis and death (Roy *et al.*, 2022; Liu *et al.*, 2020). Specifically, Treatment 3 showed a varying but persistent anthelmintic impact, indicating protracted activity that might be caused by delayed absorption or progressive bioavailability of active ingredients. As reported by Moraes *et al.* (2017), papain, a cysteine protease, suggested that such activity is due to the time requirement of the protease to fully penetrate and disrupt the structural integrity of the cuticle of nematodes. Further, the presence of benzyl isothiocyanate (BITC), a bioactive compound from the seeds of *C. papaya*, was also characterized by its gradual absorption and accumulation in target tissues, resulting in prolonged and sustained effects in test organisms (Nagesh *et al.*, 2002; Adebiyi and Adaikan, 2005). Treatment 2's (PSEE 2.5 mg/ml) reduced efficacy compared to Treatments 1 and 3, suggesting a non-linear dose-response relationship. A moderate concentration may not always translate into more significant mortality, which could be caused by differences in bioavailability, chemical breakdown, or nematode adaptability (Blanco *et al.*, 2018). Similarly, Badar *et al.* (2024) study explores *Amomum subulatum* and *Vitex negundo* at different concentrations against *Haemonchus contortus*, eliciting significant mortality rates. Higher dosages, however, did not correspondingly improve efficacy and might have adverse side effects or decreased solubility. This aligns with Goku *et al.* (2020), who found that *C. papaya* seed extract significantly reduced the time to death in *Pheretima posthuma*, even at lower concentrations, which is also supported by the presence of phytochemicals and fixed oils in the seeds that is responsible for the activity (Puangsri *et al.*, 2005).

Additionally, the delayed but decisive mortality observed in Treatment 6 may be linked to the slow-acting nature of tannins, which gradually penetrate and disrupt the nematode cuticle over time. This observation corresponds to the report of Williams *et al.* (2014), where condensed tannins from diverse plant sources significantly damage the cuticle and digestive issues of *Ascaris suum* gradually as they penetrate and disrupt the nematode's cuticle over time. Additionally, as Candra *et al.* (2024) characterized, the slow metabolism of compounds such as tannins, flavonoids, and phenolics from *C. papaya* leaf leads to prolonged effects in treating parasitic infections and sustained efficacy. These results demonstrate the possibility of papaya extracts as natural substitutes for anthelmintics. More research is necessary to optimize dosage, comprehend long-term consequences, and evaluate uses in poultry management.

Table 2. Phytochemical screening of *Carica papaya* leaves and seeds ethanolic extraction

Phytochemicals	Test	Positive result	Seed	Leaf
Tannins	Ferric Chloride test	Brownish Green or Bluish Black Color	-	-
Saponins	Foam test	Foam Formation	-	-
Flavonoids	Alkaline Reagent test	Colorless	+	-
Alkaloids	Wagner's test	Brown-Reddish Precipitate	+	-

Phytochemical screening

Table 2 shows that tannins, saponins, alkaloids, flavonoids, and glycosides were absent in PLEE, while only alkaloids and flavonoids were detected in PSEE. However, the absence of these compounds does not necessarily indicate their complete absence but rather reflects variations in phytochemical analysis, which depend on factors such as solvent type, extraction method, and test procedures (Mishra, 2024). Ying *et al.* (2021) reported that Soxhlet extraction of papaya seeds revealed the presence of these compounds using different analytical methods. Similarly, Ilham *et al.* (2019) found that papaya leaf extracts obtained with lower ethanol concentrations detected phytochemical components when alternative test methods were used.

Conclusion

The findings of this study demonstrate that *Carica papaya* seed and leaf extracts possess significant anthelmintic properties against *Ascaridia galli.* Treatment 1 (1 mg/ml seed extract), Treatment 3 (5 mg/ml seed extract), and Treatment 6 (5 mg/ml leaf extract) exhibited high mortality rates comparable to levamisole at 6 hours, highlighting their potential as alternative anthelmintic treatments. The immediate effectiveness of the seed extracts suggests a rapid mode of action, while the leaf extracts displayed a delayed but sustained effect, possibly due to differences in bioactive compound release and absorption. The non-linear dose-response observed in Treatment 2 (2.5 mg/ml seed extract) suggests that factors such as bioavailability and nematode adaptation may influence efficacy. Overall, these results support the potential use of *C. papaya* extracts as a natural alternative to conventional anthelmintics.

Recommendations

Future studies should conduct in vivo assays to verify the efficacy and safety of *Carica papaya* extracts, explore higher concentrations, and assess alternative solvents for enhanced anthelmintic potency.

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