Efficacy of *Carica papaya*, *Areca catechu*, and *Manihot esculenta* extracts against *Ascaridia galli* eggs (*In vitro*)

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ABSTRACT

Background and Objective: The anthelmintic efficacy of three plant-based extracts, such as papaya seeds, betel nut fruit, and cassava leaves was evaluated against the larval development of *Ascaridia galli* eggs, *in vitro*.

Methodology: A total of 2,000–2,500 *A. galli* eggs isolated from the intestines of native chickens were randomly distributed into 5 treatments following the complete randomized design (CRD). Treatments were: T1: piperazine, T2: phosphate-buffered saline (PBS), T3: *Carica papaya* seed extract, T4: *Areca catechu* extract, and T5*: Manihut esculenta* leaf extract. Plant-based extracts were diluted in PBS to create a dose concentration of 50 mg/mL.

Main Results: After 21 days of incubation, results revealed that extracts of *C. papaya* seed, *A. catechu*, and *M. esculenta* leaf were as effective as piperazine in inhibiting the development of *A. galli* eggs during the 7th day of incubation. *A. catechu* extract showed the highest efficacy over time, ranging from 90–100%, followed by *M. esculenta* leaf extract at 71–100%, and *C. papaya* seed extract at 51–100%. Whereas piperazine obtained 100% efficacy in controlling *A. galli* eggs throughout the experimental trial.

Conclusions: This study indicated that the extracts from *C. papaya seed, A. catechu, and M. esculenta* leaf could be utilized effectively in controlling the larval development of *A. galli* eggs as an alternative to piperazine.

Keywords: Anthelmintic, *Areca catechu, Carica papaya*, extracts, *Manihut esculenta*

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INTRODUCTION

Helminthiasis, or worm infection, caused by *Ascaridia galli* is one of the most common diseases affecting free-range backyard chickens (Ahmad *et al*., 2013). It is known to induce droopiness, hemorrhages, and diarrhea in chickens that are heavily afflicted. In addition to impairing the integrity of the intestinal mucosa, *A. galli* also interferes with the body's ability to utilize nutrients that reduce weight gain. Additionally, *A. galli* infections also result in economic losses due to treatment costs, decreased feed efficiency, and impaired performance. Traditional commercially available anthelmintic medicines, such as albendazole, mebendazole, ivermectin, piperazine, and niclosamide, which have been widely used to treat helminth infection in both humans and animals, have been linked to serious side effects such as drug resistance, food residues, and toxicity. Along with these concerns, the cost of utilizing commercial dewormers posed a significant obstacle to poultry raisers; hence, plant-based anthelmintics in poultry are now being investigated (Karumari *et al*., 2014). When compared

to commercial dewormers, these medicinal plant-based dewormers are safer, more environmentally friendly, more abundant, and less expensive to use (Feroza *et al*., 2017).

Carica papaya, Areca catechu, and *Manihut esculenta* are three of the most studied medicinal plants for their anthelmintic properties. *C. papaya* seeds, according to Olcum *et al*. (2020) are good sources of phytochemicals such as phenolics, tocopherols, phytosterols, and carotenoids which offer an array of beneficial properties from antiproliferation of cancer cells to shielding against cellular oxidative injury. It also contains phenolics, isothiocyanates, terpenes, phytosterols, flavonoids, and anthraquinones that can scavenge reactive molecules, thus protecting the cellular environment against the damaging impacts of oxidative and inflammatory activities (Enriquez-Ochoa *et al*., 2020; Wang *et al*., 2020).

A. catechu fruits from the family *Aracaceae* is a valuable anthelmintic containing flavonoid, tannins, saponins, monoterpenes, sesquiterpenes, phenols, quinones, and alkaloids (Amudhan *et al*., 2012). The arecolines and tannin found in *A. catechu* can cause paralysis, while proanthocyanidin inhibits enzymes and degrades membranes, which in turn reduces the energy production of the parasite (Mubaroka *et al*., 2019). The leaf of *M. esculenta,* as reported by Marie-Magdeleine *et al*. (2010), contains acetone, oxalic acid, saponins, and tryptophane. Depending on the variety, storage, and source of *M. esculenta* leaf, certain amounts of free quercetin, an anti-oxidative, anti-inflammatory, renal protective, and venotonic flavonoid, alkanes and sulfhydric acid, and condensed tannins (Dung *et al*., 2005) can also be traced.

Although literature data on the anthelmintic efficacy of *C. papaya*, *A. catechu*, and *M. esculenta* are available, efficacy may vary depending on the plant source and growth conditions. Therefore, the need to examine and investigate their anthelmintic effects is vital in determining optimal dosage and treatments for proper use *in vivo*. As a result, this study aimed to examine the anthelmintic effectiveness of *C. papaya* seeds*, A. catechu* fruits, and *M. esculenta* leaves extracts collected within the city of Marawi, Lanao del Sur, Philippines. Given the scarcity of research in this field, the findings here will provide significant information on the efficacy of the indicated plant-based medicinal plants in regulating *A. galli* eggs *in vitro*.

MATERIALS AND METHODS

The study was conducted at the Animal Science Laboratory, College of Agriculture, Mindanao State University, Marawi Campus from October to December 2021. The C*. papaya*, *A. catechu*, and *M. esculenta* were selected because of their known ethnomedical uses and abundance in the vicinity of Marawi, Lanao de Sur.

Preparation of the Extracts

C. papaya seeds extract was prepared following the methods described by Shaban *et al*. (2021) with slight modifications. *C. papaya seeds* were manually removed from ripe fruits, cleaned with tap water, and dried using the oven at 90 °C for 72 h. A grinder was used to smash and pulverize the dried seeds into fine powder. The powder was steeped in distilled water (1:10 w/v) for 72 h at 4 °C before being filtered using Whatman filter paper and the extraction process was repeated 3 times. The filtrates were lyophilized producing a fine sweet-smelling and chocolate color solid residue and stored in an airtight container at room temperature until use (Nwangwa, 2012).

A. catechu extract was performed according to the method described by Widiarso *et al*. (2018). Around 10 g of dried *A. catechu* was added with 300 mL of hot distilled water with constant agitation to increase solubility. The extracts were then filtered using Whatman filtered and centrifuged in 1,800 for 15 min. The filtrate was further filtered using grade No. 2 qualitative paper (Advantech MFS Inc., Dublin, CA, USA) to ensure the elimination of particles measuring ≥ 8 um.

M. esculenta extract was performed following the methods of Marie-Magdeleine *et al*. (2010). A 50 g of leaf powder was mixed into 500 mL of dichloromethane, moistened with 150 mL solvent, and left for 3 h under room temperature

without light exposure. Thereafter, the filtrate was removed and washed with solvent, and heated at 40°C. Finally, the dichloromethane extraction residue was dried under a vacuum. The extracts obtained from *C. papaya, A. catechu,* and *M. esculenta* were diluted in 50 mg/mL PBS to obtain a concentration of 50 mg/mL the same concentration as the control piperazine.

Collection of *A. galli* Worms

Specimens of adult *A. galli* worms were collected from native chickens using phosphate-buffered saline (PBS) at pH 7.4 method described by Villanueva *et al*. (2015). *A. galli* eggs were recovered by scraping the uterine portion of the parasites with a scalpel while immersed in the PBS method described by Kaingu *et al*. (2013). Eggs collected were submerged in 40 mL PBS and added with a drop of formalin (2%) to prevent mold formation.

Experimental Design and Treatment

A total of 2,000 to 2,500 *A. galli* eggs were randomly distributed into 5 treatments using the completely randomized design (CRD). Each treatment was replicated 5 times, with 100 eggs in each replicate: T1 – piperazine (a positive control), T2 – PBS (negative control), T3 – *C. papaya* seed extract, T4 – *A. catechu* extract, and T5 - *M. esculenta* leaf extract. Each sterilized petri dish contains 5 mL of fresh extracts according to treatments. These petri dishes were incubated at 26 °C under aerobic conditions (Feyera et al., 2020) for 21 days.

Data Collection

Observation of the development of *A. galli* eggs was done during the 7th, 14th, and 21st days of the incubation period under the microscope (Villanueva *et al*., 2015), while *A. galli* eggs were identified based on the drawing chart (Ackert,1931). Development of *A. galli* eggs was observed under the magnifying glass and classified into morula, tadpole, vermiform, and larval stages. While all mature infertile and infertile eggs were counted as undeveloped. The developed *A. galli* eggs were calculated using percentage (number of developed eggs divided by the total number of eggs).

Statistical Analysis

All the data gathered were processed and analyzed using a one-way ANOVA run-in SPSS version 2.0 with homogeneity of variance test. Differences among treatment means were analyzed using the least significant difference (LSD) at a 5% level of significance.

RESULTS AND DISCUSSION

Inhibition Efficiency

The average number of undeveloped eggs of *A. galli* on days 7, 14, and 21 of the incubation trial are shown in Table 1. Results revealed significant differences ($P < 0.05$) in the number of undeveloped eggs between the 5 treatments. Throughout the observation period, piperazine provides 100% efficacy in controlling the development of *A. galli* eggs. Plant-based extracts such as *C. papaya*, *A. catechu*, and *M. esculenta* were similarly found to be 100% efficient in suppressing the development of *A. galli* eggs on day 7 of incubation. Furthermore, PBS inhibits only 68.59% of the time on the same day of observation.

On the 14th day, reduced inhibition efficiency from the natural plant extract was observed. Among the natural extracts, *A. catechu* obtained the maximum efficiency of 94.50%, followed by *M. esculenta* extracts at 83.78%, and *C. papaya* at 74.78%. PBS had the lowest effectiveness at 46%. On the final day of observation (the 21st day after incubation), *A. catechu* extract still showed the best effectiveness in suppressing *A. galli* eggs with 89.96%, followed by *M. esculenta* leaf extract with 70.59% and *C. papaya* with 50.64%. PBS, on the other hand, was just 46.33% effective. The results herein indicate that *C. papaya, A. catechu*, and *M. esculenta* extract can effectively control the development of *A. galli* eggs. The effectiveness shown by the plant-based extracts in controlling *A. galli* eggs could be attributed to their anthelmintic properties such as flavonoids, tannins, alkaloids, saponins, and other phytocomponents that affect internal parasite activities (Olagunju *et al*., 2009). According to Greiffer *et al*. (2022), tannin, which is commonly found in plant-based medicinal plants,

binds to the cuticle of nematodes, and causes disruption of the cuticle structure leading to molting impairment and locomotion defects. Lorent *et al*. (2014) also added that the cytotoxic activity of saponins can form pores on cell membranes which disrupt the ionic balance of the cell, resulting in lysis and death. Alkaloids are also found to inhibit transport across cell membranes (Nyambuya *et al*., 2017). In the current investigation, *A. catechu* displayed the maximum efficacy in suppressing *A. galli* compared to *C. papaya* and *M. esculenta* with 90% effectiveness at 21 days of incubation. *A catechu* extract, as reported by Amudhan *et al*. (2012), contains 20% polyphenols, of which flavanols are 10% and epicatechin is 2.5%. Major alkaloids isolated are arecoline which constitutes 7.5 mg/g weight, arecaidine at 1.5 mg/g weight, guvacoline at 2.0 mg/g, and guvacine at 2.9 mg/g weight. When compared to *C. papaya* seed extract and *M. esculenta* extract, these chemical compounds provided *A. catechu* with a substantially better strategy for limiting *A. galli* proliferation. Moreover, in the current investigation, *C. papaya*, and *M. esculenta* extracts remained beneficial after 21 days of incubation, albeit with lower efficacy of 50.64% and 70.59%, respectively. These findings are consistent with the results obtained by several authors regarding the positive anthelmintic effects of *C. papaya* seed extract (Aravind *et al*., 2013; Dakpogan *et al*., 2019; Nghonjuyi *et al*., 2020), *A. catechu extract* (Begum *et al*., 2010; Raza *et al*., 2016; Mubaroka *et al*., 2019), and *M. esculenta* leaf extract (Nguyen *et al*., 2003; 2005; Sokerya and Preston, 2003).

Table 1 Total mean of undeveloped *Ascaridia galli* eggs during the 7th, 14th, and 21st day of incubation

Treatments	Day of incubation		
	Day 7	Day 14	Day 21
T1: Piperazine	100.00 ± 0.34[a]	100.00 ± 0.12[a]	100.00 ± 0.43[a]
T2: Phosphate-buffered saline	68.59 ± 6.68[b]	46.00 ± 3.81[c]	46.33 ± 6.68[c]
T3: *C. papaya* seed extract	100.00 ± 0.23[a]	74.78 ± 3.01[b]	50.64 ± 3.39[c]
T4: *A. catechu* extract	100.00 ± 0.65[a]	94.50 ± 2.30[a]	89.96 ± 3.33[b]
T5: *M. esculenta* leaf extract	100.00 ± 1.12[a]	83.78 ± 5.03[b]	70.59 ± 4.20[b]

Note: [a,b,c] Means within columns having the different superscripts indicates significant difference based on the least significant difference at $P < 0.05$

Percentage of Developed *A. galli* Eggs

Table 2 shows the percentage of developed *A. galli* eggs from the various treatments. The results showed that inoculating *A. galli* eggs in PBS produced the most developed eggs compared to the plant-based medicinal treated groups. Poly-(butylene succinate) as the acronym of PBS does not possess the same anthelmintic compounds as *C. papaya, A. catechu*, and *M. esculenta,* the very reason it only provided less efficiency in controlling *A. galli* eggs. PBS is a pH-adjusted blend of ultra-grade phosphate buffers and saline solutions, that contains 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 2 mM KH_2PO_4. The mechanisms of how PBS these components affect *A. galli* proliferation are not yet known. Factors such as oxygen (Ackert, 1931), temperature, and humidity (Reid, 1960; Permin and Hansen, 1998) are known to affect the larval development of *A. galli* eggs.

Table 2 Degree of developed *Ascaridia galli* eggs during the 7th, 14th, and 21st days of the incubation period

Treatments	Day of incubation			
	Day 0	Day 7	Day 14	Day 21
T1: Piperazine	100% UE	100% UE	100% UE	100% UE
T2: Phosphate-buffered saline	100% UE	69% UE 31% VS	46% UE 20% EMS 34% TS	46% UE 13% EMS 20% LMS 21% TS
T3: *C. papaya* seed extract	100% UE	100% UE	74% UE 26% VS	51% UE 23% VS 18% LS 8% EMS
T4: *A. catechu* extract	100% UE	100% UE	95% UE 5% VS	90% UE 10% VS
T5: *M. esculenta* leaf extract	100% UE	100% UE	84% UE 16% VS	71% UE 17% VS 12% LS

Note: UE = unfertilized eggs, VS = vermiform stage, LS = larvae stage, EMS = early morula stage, LMS = late morula stage, TS = tadpole stage.

During the 14th day of incubation, results revealed that all the developed *A. galli* eggs from the plant-based extracts were categorized in the vermiform stage except for PBS, with 34% tadpole and 20% early morula stage. The fewest developed eggs were noted in *A. catechu* (5% vermiform stage), followed by *M. esculenta* leaf extract (16% vermiform stage), and *C. papaya* seed extract (26% vermiform stage). On the final day (21st day) of observation, *A. catechu* extract consistently showed the fewest number of developed *A. galli* eggs (10% vermiform stage). On the other hand, approximately 29% developed *A. galli* eggs were found in eggs treated with *M. esculenta,* with 17% identified in vermiform stage and 12% in the larva stage. Similarly, 49% developed *A. galli* eggs have also been observed in *C. papaya* seed extracts treated groups. There were 23% vermiform stage, 18% larva stage, and 8% early morula stage. The lowest efficacy during this time was noted in *A. galli* eggs treated with PBS with only 46% undeveloped eggs. Approximately 54% of *A. galli* eggs in this treatment progressed into early morula stage (13%), late morula stage (20%), and tadpole stage (21%).

This current result suggests that despite the shown percentages of developed eggs, the use of *A. catechu, C. papaya*, and *M. esculenta* extracts can effectively inhibit the development of *A. galli* eggs. Moreover, the reduced efficiency observed over time from the indicated plant-based extract can be associated with the variations of anthelmintic components found in each plant. Fajrin and Tunjung (2014) reported that the flavonoid content of the plant varies depending on cultivar, environmental factors, and production practices (Singh and Ali, 2011; Laya and Koubala, 2020). On the other hand, Goku *et al*. (2020) observed that the effects of *C. papaya* extracts on paralysis and death times were concentration dependent. They further reported that increasing the concentration of the extract from 1 mg/mL to 5 mg/mL caused a significant decline in the time of paralysis of the

Pheretima posthuma. Similar observations were also reported by Rupa and Jayanta (2013), Mintah *et al*. (2017), and Ameen *et al*. (2018).

CONCLUSION

Based on the above-presented results, it is therefore concluded that the plant-based extracts used in the study such, as *A. catechu* fruit, *C. papaya* seed, and *M. esculenta* leaf, at a rate of 50 mg/mL, can control the development of *A. galli* eggs, *in vitro*, providing a 100% efficacy rate at 7 days of incubation period. All the indicated extracts can inhibit *A. galli* eggs up to 21 days of incubation with a maximum efficiency of 90% for *A. catechu*, 71% for *M. esculenta*, and 51% for *C. papaya* seeds. Therefore, the indicated plant-based extract can be used effectively as an alternative to commercial anthelmintic for poultry production.

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