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# *In vitro* evaluation of jackfruit (*Artocarpus heterophyllus*) seed ethanolic extract as an anthelmintic against *Ascaridia galli* in native chickens

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**ABSTRACT**

Helminth infections, particularly *Ascaridia galli*, significantly impair poultry health and productivity, especially among native chickens raised under natural conditions. The continuous of use of synthetic anthelmintics have led to resistance and drug residue concerns, emphasizing the need for natural alternatives. This study aimed to evaluate the *in vitro* anthelmintic activity of jackfruit (*Artocarpus heterophyllus*) seed ethanolic extract against *Ascaridia galli* and compare its efficacy with albendazole. A Completely Randomized Design (CRD) was employed with six treatments, 3 concentrations of albendazole (25, 50 and 100mg/5ml of distilled water) and 3 concentrations of jackfruit seed ethanolic extract (25, 50 and 100µl/5ml distilled water) replicated three times. Adult *Ascaridia galli* worms were exposed to each treatment, and observations on paralysis and mortality were recorded every 30 minutes. Paralysis was occurred as early as 1:30 hours with complete mortality recorded at 3 hours. The 100mg albendazole and 100µl of jackfruit seed ethanolic extract treatments showed the highest mortality, while 25µl jackfruit seed ethanolic extract exhibited comparable activity to 25mg albendazole, yielding significant paralysis (3.67) and mortality (5.67 values). Results indicate that jackfruit seed ethanolic extract possesses potent, rapid and dose dependent anthelmintic activity against *Ascaridia galli*. The 25µl/5ml of distilled water concentration was identified as minimal effective and potentially safe dose. Therefore, jackfruit seed ethanolic extract represent a promising, affordable, and ecofriendly natural deworming alternative for sustainable poultry production.

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## INTRODUCTION

Gastrointestinal parasites infections remain one of the major problems limiting productivity among smallholder farmers. These parasites affect the animal's ability to utilized feed efficiently by disrupting digestion and nutrient absorption. As a results, infected animals often suffer from poor growth, weight loss, anemia, and other health issues caused by protein depletion and mineral imbalances (Holmes, 1987). For many years, chemical dewormers have been the main solution for controlling their parasites. However, the growing problem of anthelmintic resistance, particularly in Southeast Asia and the Philippines, has raised concerns about the long-term effectiveness of these drugs (Ventura, 2003). These challenges led researchers and framers to explore alternative, more sustainable, more suitable control methods. One promising approach is the use of plant-based dewormers, which are often affordable, environmentally friendly and locally available. Among the plants with potential anthelmintic properties are jackfruit (*Artocarpus heterophyllus)* and *Tamarindus indica* L. both of which are commonly found in tropical regions. While these plants have shown promise in traditional medicine, their effectiveness against poultry parasites has not been fully studied. One of the most common and harmful intestinal parasites in chicken is *Ascaridia galli,* a nematode that spreads easily through contaminated soil and feed. Infections can cause diarrhea, anemia, poor weight gained, reduced egg production and in severe cases intestinal blockage or secondary infections (Soulsby, 1982; Mlondo *et al.*, 2022; Hoglund *et al.*, 2023). Because of this widespread occurrence and negative effects on poultry and productivity, controlling *Ascaridia galli* is essential. This study aimed to contribute to that goal by exploring the potential of jackfruit seed ethanolic extract (JSEE) as natural dewormers. Specifically, it evaluates the *in vitro* anthelmintic activity of JSEE against *Ascaridia galli* and compare its efficacy with albendazole. The findings of this research may help develop cost effectiveness and suitable parasites control strategies for poultry. Ultimately benefiting smallholder farmers and promoting more resilient livestock production.

## MATERIALS AND METHODS

### Plant identification

The plant specimen, *Artocarpus heterophyllus* (Jackfruit) seeds, was authenticated by the Biodiversity, Environment, and Natural Resources Research Centre, Cebu Technological University, Argao Campus.

### Collection of jackfruit seed

Mature jackfruit (*Artocarpus heterophyllus)* seeds were collected from Barangay Camingawan, Kabankalan City, Negros Occidental, Philippines. The seeds were thoroughly washed with clean water to remove any surface dirt or contaminants, the air dried at room temperature for fifteen (15) days. Once completely dried, the seeds were ground into fine powder using manual grinder. The powdered sample was stored in airtight containers to prevents moisture absorption until further use in the extraction process.

### Extraction procedure

The ethanolic extraction procedure was carried out following the methods of Peter *et al.* (2014), Owoyele *et al.* (2008), Masfufatun *et al.* (2018), Gulpan *et al.* (2025) and Abrasa *et al.* (2025) with slight modifications. Approximately 750 grams of powdered jackfruit seed was soaked (macerated) in 2,800 ml (95% ethanol) for 72 hours at room temperature with gentle stirring time to time. After maceration, the mixture was filtered through Whatmann No. 1 filter paper to remove solid residues. The filtrate was then concentrated using a rotary evaporator at 40°C and 104 rpm under reduced pressure. The results extract was labelled and stored at 20°C until used for phytochemical and biological analysis.

### Phytochemical screening

Qualitative phytochemical screening of the *Artocarpus heterophyllus* seed ethanolic extract was performed at Applied Microbiology Laboratory, Faculty of Biology, University of San Carlos-Talamban Campus. Standard phytochemical methods

were used to detect the presence of major secondary metabolites such as, alkaloids, saponins flavonoids, steroids, and terpenoids. The presence of each compound was confirmed through characteristic color changes of the formation of precipitates, which indicated a positive reaction (Table 1).

**Table 1.** Results of phytochemical analysis of jackfruit seed ethanolic extract

| Phytochemical analysis | Method | Positive results | Extract |
|---|---|---|---|
| | | | Jackfruit seed |
| Flavonoids | Alkaline reagent test | Colorless | - |
| Steroids | Liebermann Burchard test | Green/Blue-green color | - |
| Saponins | Foam test | Foam formation | - |
| Alkaloids | Wagner's test | Brown-reddish precipitate | + |
| Terpenoids | Salkowski test | Reddish brown color at the interface | + |

**Collection and identification of *Ascaridia galli***

Adult female *Ascaridia galli* worms were collected from the intestine of freshly slaughtered native chickens at a private slaughterhouse in Dumanjug Cebu, Philippines. The collected worms were carefully wash with Normal Saline Solution (0.9% NaCl) to remove debris and intestinal content, then placed in sterile containers for transport to laboratory. Morphological identification was submitted to the College Veterinary Medicine, Visayas State University (VSU), Baybay Leyte. Identification was done as using stereomicroscope. Based on the key diagnostic features, the worms were confirmed as *Ascaridia galli,* characterized by elongated, cylindrical bodies with semitransparent yellowish-white cuticle. The anterior end was surrounded by the three prominent lips-one dorsal and two subventrals. All specimens examined were female, possessing blunt, rounded tails and measuring between 85-94m in length.

***In vitro* anthelmintic assay**

The collected mature female worms from freshly slaughtered native chickens were carefully cleaned with 0.9% normal saline solution to remove any debris and intestinal contents. Each 100-mm Petri dish contained 10 live worms, and varying volumes of jackfruit seed ethanolic extract (25, 50 and 100µl/5ml distilled water) were administered as treatment solutions. The positive control consisted of albendazole at concentrations of 25, 50 and 100mg/5ml distilled water. The petri dishes were maintained at 37°C, and the worms' movement was observed every 30 minutes. Worms that exhibited no movement under mechanical stimulation were classified and dead.

**Experimental design and data analysis**

A Completely Randomized Design (CRD) was employed to assess the *in vitro* effectives of the *Artocarpus heterophyllus* seeds ethanolic extract against *Ascaridia galli.* The experiment had six (6) treatments, replicated three (3) times with ten (10) worms per replicate. Each petri dish consisted of ten (10) worms, for a total of 180 worms throughout all treatments. The observations for the number of paralyzed and killed worms were made every 30 minutes.

**Statistical analysis**

Data collected on worm paralysis and mortality were summarized as means and analyzed using STAR (Statistical Tool for Agricultural Research) software, developed by the International Rice Research Institute (IRRI, Los Baños, Philippines). One-way Analysis of Variance (ANOVA) was performed to determine significant differences among treatments. Mean separations were carried out using Tukey's Honest Significant Difference (HSD) test at a 1% level of significance ($p < 0.05$).

**RESULTS AND DISCUSSION**

**Mean paralyzed worm in different time exposure**

The Table 2 below present the mean number of paralyzed *Ascaridia galli* worms were recorded under varying concentrations of jackfruit seed ethanol extract

(JSEE) and albendazole to evaluate the extract's speed action and effectiveness relative to a standard anthelmintic drug. Paralysis was observed only during 1 hour and 30 minutes exposure period, while no paralyzed worms were detected at 2:30 and 3 hours. This indicate that paralysis occurred early in the exposure, and by the later stages, most worms had already died. Such a pattern reflects the rapid anthelmintic activity of both albendazole and JSEE.

Among the treatments, T1 (25mg of albendazole/5ml of distilled water and T4 (25µl JSEE/5ml distilled water) produced the highest mean number of paralyzed worms (3.67), showing a strong and immediate inhibition of motility. This was followed by (50µl JSEE/5ml distilled water) with 3.00, and T2 (50mg albendazole/5ml distilled water) with 2.33 paralyzed worms. The lowest number of paralyzed worms (1.00) was observed in T3 (100mg albendazole/5ml distilled water) and T6 (100µl of JSEE/5ml distilled water), indicating that higher concentrations caused faster worm death, leaving less time for paralysis to occur. This pattern reflects a dose-dependent effect, where increasing the extract concentration shortens the interval between paralysis and mortality.

**Table 2.** Mean paralyzed worm in different time exposure

| Treatments | Observation period | | |
|---|---|---|---|
| | 1:30 hrs. | 2:30 hrs. | 3:00 hrs. |
| T1- 25mg albendazole/5ml distilled water | 3.67[a] | 0 | 0 |
| T2- 50mg albendazole/5ml distilled water | 2.33[b] | 0 | 0 |
| T3- 100mg albendazole/5ml distilled water | 1.00[c] | 0 | 0 |
| T4- 25µl of JSEE/5ml distilled water | 3.67[a] | 0 | 0 |
| T5- 50µl of JSEE/5ml distilled water | 3.00[ab] | 0 | 0 |
| T6- 100µl of JSEE/5ml distilled water | 1.00[c] | 0 | 0 |
| Mean | 2.44 | 0 | 0 |
| CV (%) | 16.70% | 0 | 0 |
| t-test | ** | 0 | 0 |

The findings confirm that the ethanolic extract of jackfruit seed exhibits strong anthelmintic properties comparable to the synthetic drug albendazole, particularly at a concentration of 25µl of JSEE/5ml distilled water. This observation supports the report of Yashaswini *et al.* (2016), who found a similar dose-dependent trend in *Pheretima posthuman*. The activity of JSEE can be attributed to its phytochemical contents such as alkaloids, flavonoids, and tannins which are known to interfere with the neuromuscular function of parasites, leading to paralysis and death (Martin, 1985; Mali, 2007; Roy *et al.*, 2010; Ranasinghe *et al.*, 2023). Similar mechanism has been described in other medicinal plants like neem (*Azadirachta indica)* and cashew (*Anacaridum occidentale),* where tannins and phenolic compounds caused neuromuscular disruption and eventual paralysis (Verkerk *et al.,* 1993; Aiswarya *et al.*, 2011). In addition, condensed tannins from forage plants have been reported to reduce larval survival and inhibit egg hatching in nematode (Min and Hart, 2003). These similarities suggest that JSEE functions through comparable biochemical pathways, reinforcing its potential as an effective botanical anthelmintic. Overall, the results demonstrate that jackfruit seed ethanolic extract acts against *Ascaridia galli* and performs comparable to albendazole even at lower concentrations. The optimal dose ((25µl JSEE/5ml distilled water) provides an ideal balance between potency and exposure duration, making it both practical and cost effective for small scale poultry operations. Beyond its efficacy, JSEE presents a sustainable and eco-friendly alternative to synthetic dewormers, offering smallholder farmers an affordable solution that may reduce drug resistance and support organic poultry production in resource-limited areas.

**Mean mortality of worms in different time exposure**

Table 3 below present was the mortality rate of *Ascaridia galli* increased consistently with both higher

concentrations and longer exposure times of the jackfruit seed ethanolic extract (JSEE) and albendazole. The highest mortality was observed in the T6 (100µl of JSEE/5ml distilled water), where all worms died after only 2:30 hours of exposure, followed by the T5 (50µl of JSEE/5ml distilled water) and T4 (25µl of JSEE/5ml distilled water), which also achieved 100% mortality but at slightly longer intervals.

Among the positive controls, albendazole at T3 (100mg/5ml distilled water) recorded complete worm mortality within 1:30 hours, demonstrating its rapid pharmacological action. Notable JSEE at T6 (100µl of JSEE/5ml distilled water) exhibited a similar mortality response, indicating that the extracts bioactive compounds were highly potent event at relatively low concentrations. The progression from paralysis to death across treatments supports a dose-and time dependent anthelmintic effect, where higher concentrations of JSEE accelerated worm mortality due to stronger neuromuscular interference.

**Table 3.** Mean mortality of worms in different time exposure

| Treatments | Observation period | | |
|---|---|---|---|
| | 1:30 hrs. | 2:30 hrs. | 3:00 hrs. |
| T1- 25mg albendazole/5ml distilled water | 6.00[b] | 4.00[a] | 0 |
| T2- 50mg albendazole/5ml distilled water | 6.33[b] | 3.67[a] | 0 |
| T3- 100mg albendazole/5ml distilled water | 9.00[a] | 1.00[b] | 0 |
| T4- 25µl of JSEE/5ml distilled water | 5.67[b] | 4.33[a] | 0 |
| T5- 50µl of JSEE/5ml distilled water | 6.00[b] | 4.00[a] | 0 |
| T6- 100µl of JSEE/5ml distilled water | 9.00[a] | 1.00[b] | 0 |
| Mean | 7.00 | 3.00 | - |
| CV (%) | 4.76% | 3% | - |
| t-test | ** | ** | |

The observed mortality pattern agrees with earlier report of Yashaswini *et al.* (2016), found that ethanolic extracts from jackfruit seeds induced both paralysis and death in *Pheretime posthuman* in a concentration-dependent manner. The efficacy of the extract can be attributed to secondary metabolites such as tannins, alkaloids, saponins and flavonoids, which are known to disrupt the parasite's energy metabolism and inhibit acetylcholine esterase activity (Roy *et al.*, 2010; Ranasinghe *et al.*, 2023). Similarly, studies on other botanical anthelmintics such as *Areca catechu* (betle nut), *Azadirachta indica* (neem) and *Tamarindus indica* (tamarind leaves)- have demonstrated comparable bioactivity. These plants compounds cause cuticular damage and ionic imbalance in nematodes, leading to irreversible paralysis and dead (Mali and Wadekar, 2008; Verma *et al.*, 2011; Singh *et al.*, 2018). The rapid mortality indicates that the phytochemical constituents of JSEE likely act through multiple mechanism-neurotoxicity, inhibition of motility, and interference with parasite metabolism-making it a strong natural alternative to albendazole.

The findings establish that jackfruit seed ethanolic extract is highly effective in inducing mortality in *Ascaridia galli,* performing comparably to albendazole within a similar timeframe. This reinforces its potential as a promising herbal anthelmintic, particularly in a small-scale poultry operation where access to commercial deowormer is limited. Utilizing jackfruit seed waste for medical purposes not only adds economic value to agricultural by-products but also supports sustainable and eco-friendly parasite control. The extract's fast-acting and dose-dependent response further suggests its potential for formulation into standardized natural deworming preparations, reducing dependency on synthetic drugs and mitigating issues of drug resistance in poultry nematodes.

## CONCLUSION

This study found that the ethanolic extract of jackfruit seeds (JSEE) showed strong anthelmintic activity against *Ascaridia galli,* comparable to albendazole. The 25µl JSEE/5ml distilled water concentration proved most effective, causing rapid paralysis and death worms.

Its activity is likely due to phytochemicals such as alkaloids, flavonoids, tannins and saponins that disrupt the parasite's neuromuscular function. These results suggest that JSEE could serve as natural, affordable, and eco-friendly alternative to synthetic dewormers, benefiting small holder poultry farmers while promoting sustainable use of agricultural by-products. Further studies are recommended to verify its safety and optimize its application in field conditions.

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