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In vitro anthelmintic activity of *Areca catechu* (Betel nut) seed ethanolic extract against (*Ascaridia galli*) in native chickens

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Abstract

Ascaridia galli is a common intestinal parasite in chickens, causing significant health issues and economic losses. Conventional anthelmintics are expensive and prone to resistance, necessitating the search for alternative treatments. This study evaluated ethanolic betel nut (*Areca catechu*) seed extract (BNSEE) kills *A. galli* in a lab setting. Five treatments were used on nematodes that had just been collected from native chickens that had been slaughtered: T0 (levamisole, positive control), T1 (1 ml BNSEE), T2 (1.5 ml BNSEE), T3 (2 ml BNSEE), and T4 (2.5 ml BNSEE). Worm mortality was assessed at 1, 3, and 6 hours. Statistical analysis ($p < 0.05$) revealed significant differences among treatments, while time interval and treatment × time interaction effects were not significant. The highest death rates were seen in T0 (10%) and T4 (9.33), but there was no significant difference between them. This suggests that BNSEE at 2.5 ml has comparable anthelmintic activity to levamisole. Lower BNSEE concentrations (1–2 ml) resulted in significantly lower mortality rates, demonstrating a dose-dependent response. Phytochemical analysis confirmed the absence of tannins, saponins, alkaloids, flavonoids, and glycosides in betel nut seeds. These findings suggest that BNSEE is a surprising due to the alkaloids in betel nuts. Further, *in vivo* studies are recommended to evaluate its safety, efficacy, and potential application in poultry parasite management.

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Introduction

Ascaridia galli, a common intestinal parasite, poses a significant threat to poultry production worldwide. Severe infections can damage intestinal villi (Salam *et al.*, 2015; Alrubaie, 2015), alter mucosal defence (Darmawi *et al.*, 2012), and reduce nutrient absorption, leading to poor weight gain (Das *et al.*, 2010). This parasite has been widely reported in countries such as Egypt (Hassanain *et al.*, 2009), Bangladesh (Begum *et al.*, 2010; Saha *et al.*, 2015), Germany (Kaufmann *et al.*, 2011), and India (Ahmad *et al.*, 2013). The economic losses caused by *A. galli* are substantial, affecting both backyard and commercial poultry farms. To manage these infections, commercial anthelmintics are commonly used. However, these treatments have drawbacks, including environmental pollution, adverse effects on host animals, and rising resistance among parasites (Vercruysse *et al.*, 2011). Anthelmintic resistance has been observed in various livestock species, such as Colombian sheep (Garcia *et al.*, 2016), goats in Pakistan (Saeed *et al.*, 2007), and cattle in Brazil (Soutello *et al.*, 2007). Resistance to ivermectin and benzimidazole has also been reported in Germany, Belgium, and Sweden, leading researchers to recommend more extensive anthelmintic efficacy testing (Demeler *et al.*, 2009). Due to these challenges, plant-based anthelmintics have gained attention as potential alternatives. Betel nut (Areca catechu) contains bioactive compounds such as tannins and alkaloids, which have shown anthelmintic properties. However, its effectiveness against *A. galli* remains unclear. This study aims to assess the efficacy of ethanolic betel nut seed extract against *A. galli* in an *in vitro* setting. The findings will contribute to the growing body of knowledge on plant-based dewormers and their potential applications in veterinary medicine and poultry health management.

Materials and methods

Ethical consideration

The study followed all the rules set by the Bureau of Animal Industry (BAI) in Manila, and the Institutional Animal Care and Use Committee (IACUC) at Cebu Technology University-Main Campus gave its approval. Betel nut seeds (*Areca catechu*) were authenticated by Prof.

Hemres Alburo at the Biodiversity, Environment, and Natural Resources Research Centre, Cebu Technological University—Argao Campus.

Materials

Beetle nut seed, 95% ethanol, a blender, a rotary evaporator (for concentration), Petri dishes, a mortar and pestle, regular saline solution, a micropipette, an incubator, a levamisole, and an empty maceration container are the items used in this investigation.

Extraction procedure

The procedure of Peter *et al.* (2014) was employed to collect fresh betel nut seeds, thoroughly cleanse them, and thereafter allow them to air dry for 15 days at ambient temperature. It recovered the desiccated components via cold maceration after pulverization and storage in airtight containers. A new method from Owoyele *et al.* (2008) and Masfufatun *et al.* (2018) was used to soak 750 g of Areca catechu powder in 2,800 ml of 95% ethanol for 72 hours at room temperature. Used a circulating water vacuum pump SHZ-DIII and a Rotary Evaporator RE-100 Pro (Biobase, China) to concentrate the extracts at 40°C and 104 rpm. I filtered the concentrated extracts using Whatman No. 1 filter paper, tagged them, stored them at -20°C for subsequent analysis, and then preserved them.

Phytochemical screening

A qualitative phytochemical screening of betel nut seed extracts was conducted on the applied microbiology campus of the Faculty of Biology at the University of San Carlos-Talamban Campus. This analysis was conducted by Sir Carl Raymundo Consuegra, the project's technical assistant II and noted Dr. Jonie C. Yee chairman of the Department of Biology, used a variety of test methods to confirm the presence of flavonoids, tannins, saponins, alkaloids and glycosides (Table 1).

Table 1. Phytochemical screening of betel nut seed ethanolic extraction

Phytochemical analysis	Method	Positive results	Extract
			Areca nut
Flavonoids	Alkaline reagent test	Colorless	-
Tannins	Ferric chloride test	Brownish of bluish black color	+
Saponins	Foam test	Foam formation	-
Alkaloids	Wagner's test	Brown-reddish precipitate	+
Glycosides	Keller-Killiani test	Brown-ring color at the interface	_

In vitro anthelmintic assay

Ascaridia gali worms come from the intestines from recently slaughtered indigenous chickens in the private slaughterhouse in Dumanjug, Cebu, Philippines. They were washed, incorporated into NSS (0.9% sodium chloride) and brought into the laboratory. Their viability and motility were confirmed following the method of Amelia *et al.* (2017). After counting, the nematodes are divided into five groups and kept at 37°C (Aziz *et al.*, 2018). The worms were gently generated with tweezers to assess mortality. Those who did not move were called dead or unmoved. The efficacy of ethanol extracts in vitro was determined by determining the average mortality rate of motivated worms after exposure (Cabardo *et al.*, 2017). Each 100 mm Petri dish was assigned a test extract to ten worms. The experiment consisted of five treatments containing To (positive control) in which the worms were exposed to levamisole and four test treatments (T1, T2, T3, and T4), 1 mL of worms, 1.5 mL, 2 mL, or 2.5 mL of betel nut seed ethanolic extract (BNE). Each treatment is repeated three times and has a fully randomized design (CRD) with two factor configurations. Factor A was the concentration of BNE (four levels), and Factor B was the exposure time (e.g., 1 hour, 3 hours, 6 hours). Due to reduced worm mortality, the anthelmintic effect was assessed over time. Six hours later, the extracts were washed away and warm NS (0.9% sodium chloride) worms were detected for 30 min to assess motility revival.

Morphological verification and certification of parasites

Worms collected at the Veterinary Medical School of Visayas State University (ViSCA) were identified by Professor Harvey P. Portugaliza. Identification was conducted through the examination of key morphological features under a stereomicroscope to ensure accurate species confirmation.

Morphological analysis

Based on the analysis conducted by Professor Harvey P. Portugaliza at the Faculty of Veterinary Medicine, Visayas State University (ViSCA), three nematode samples, measuring 85 mm, 94 mm, and 88 mm, were collected from a chicken. Each nematode exhibited an elongated, cylindrical body that was semitransparent and yellowish-white, covered by a cuticle. The body tapered toward the anterior end, which featured a mouth surrounded by three distinct lips: one dorsal lip and two subventral lips. All specimens were female, characterized by blunt, rounded tails.

Results and discussion

Mortality rate of Ascaridia galli at different BNSEE concentrations

Table 2 presents the mean mortality of *Ascaridia galli* exposed to different concentrations of betel nut seed ethanolic extract (BNSEE) at 1-hour, 3-hour, and 6-hour time intervals. The results indicate that To (1 ml levamisole, positive control) and T4 (2.5 ml BNSEE) exhibited the highest mean mortality rates, with 10.00 and 9.33 respectively, showing no statistically significant difference ($p > 0.05$). Lower concentrations of BNSEE (1 ml, 1.5 ml, and 2 ml) resulted in significantly lower mortality rates ($p < 0.05$), suggesting a dose-dependent response.

Effect of exposure time on worm mortality

At the 1-hour observation, To (10.00) and T4 (9.67) recorded the highest mortality, while T1 (1 ml BNSEE) showed the lowest mortality (3.67). By 3 hours, mortality remained highest in To (10.00 ± 0.00) and T4 (9.00 ± 1.00), while T1 increased

slightly to 4.67 At 6 hours, T0 maintained complete mortality (10.00), while T4 slightly increased to 9.33 ± 0.58. The lowest mortality remained in T1 (3.33), followed by T2 (1.5 ml BNSEE, 4.00) and T3 (2 ml BNSEE, 5.00).

Statistical analysis

Statistical analysis using LSD at $p < 0.05$ confirmed that T0 (levamisole) and T4 (2.5 ml BNSEE) were not significantly different, indicating comparable anthelmintic activity. The interaction between concentration and time ($p > 0.05$) was not significant, suggesting consistent mortality patterns across time intervals. The coefficient of variation (CV = 17.67%) indicates moderate variability in the dataset.

Comparison with commercial anthelmintics

These findings reinforce the efficacy of BNSEE as an alternative to synthetic anthelmintics. Riviere *et al.* (2013) highlighted that anthelmintics like levamisole and fenbendazole are widely used in poultry due to their effectiveness in eradicating parasitic worms without negatively affecting egg production. Similarly, Ebrahimi *et al.* (2014) and Eslami *et al.* (2009) emphasized the impact of untreated *A. galli* infections in free-ranging hens, resulting in weight loss and reduced egg production. Given these challenges, the need for sustainable, plant-based dewormers is evident.

Bioactive compounds responsible for anthelmintic activity

The phytochemical composition of BNSEE supports its anthelmintic potential. Roy *et al.* (2022) and Liu *et al.* (2020) reported that bioactive compounds such as alkaloids, flavonoids, tannins, and proteolytic enzymes like papain interfere with nematode neuromuscular function, leading to paralysis and death. Wang *et al.* (2014) identified arecoline, arecolidine, guvacoline, guvacine, and isoguvacine as major alkaloids in betel nuts. Sharma *et al.* (1997) further confirmed that arecoline is the primary alkaloid responsible for anthelmintic effects by inducing temporary paralysis in worms.

Table 2. Mean mortality of *A. galli* exposed to varying concentrations of betel nut seed extract at different observation periods

Treatment	Time interval			
	1 hour	3 hours	6 hours	Mean
T0-1 ml levamisole Control	10	10	10	10[a]
T1-1 ml BNSEE	3.67	4.67	3.33	3.89[c]
T2-1.5 ml BNSEE	6.33	6.00	4.00	5.44[b]
T3-2 ml BNSEE	6.67	6.67	5.00	6.11[b]
T4-2.5ml BNSEE	9.67	9.00	9.33	9.33[a]
Mean	7.26	7.27	6.33	6.95
C × TI	ns			
CV (%)	17.67%			

Means with the same letter are not significantly different.

Treatment 0 - worms were exposed to 1ml Levamisole, Treatment 1 - worms were exposed to 1ml BNEE, Treatment 2 - worms were exposed to 1.5ml BNEE, Treatment 3 - worms were exposed to 2 ml BNEE

In addition, tannins in ethanol extracts of betel nuts have been shown to act as anthelmintics by inhibiting enzymatic activity and damaging the nematode membrane, leading to nutrient deprivation and eventual death (Shahidi *et al.*, 1995). Flavonoids in BNSEE also play a crucial role in disrupting metabolism and oxidative balance, further contributing to worm mortality (Saxena *et al.*, 2013; Poolperm *et al.*, 2017).

Dose-dependent effect and comparison with other plant-based anthelmintics

The dose-dependent efficacy of BNSEE aligns with previous studies on plant-derived dewormers. Suleiman *et al.* (2014) and Sarojini *et al.* (2011) reported that methanol extracts of *Cassia occidentalis* and *Guiera senegalensis* exhibit greater efficacy at higher concentrations due to the presence of tannins,

flavonoids, and saponins. Similarly, Ali *et al.* (2011) and Thanh *et al.* (2023) demonstrated that increasing the concentration of alkaloids and flavonoids from *Saraca indica* significantly enhanced helminth mortality. Furthermore, research by Molina *et al.* (2007) and Yamson *et al.* (2019) confirmed that betel nut extracts exhibit strong anthelmintic activity, with ethanol extracts causing complete immobilization of *Fasciolla* species. Jeyathilakan *et al.* (2010) validated that BNSEE with the higher concentrations effectively eliminated *Fasciola* spp. Within minutes, reinforced its potential as a natural dewormer.

Potential limitations and future directions

While these findings demonstrate the potential of BNSEE as an effective anthelmintic alternative, certain limitations must be considered. The current study was conducted in vitro, and results may differ in in vivo conditions due to variations in parasite susceptibility, host metabolism, and environmental factors. Further in vivo studies are necessary to assess BNSEE's safety, optimal dosing, and long-term effects in poultry production. Additionally, while BNSEE showed promising efficacy at higher concentrations, its practicality in large-scale poultry farming requires further investigation. The economic feasibility and potential side effects on chickens should be explored to determine whether BNSEE can replace or complement existing commercial anthelmintics.

Conclusion

This study investigated the anthelmintic potential of ethanolic betel nut seed extract (*Areca catechu*) against *Ascaridia galli* in an *in vitro* setting. The results showed that the highest concentration (2.5 ml) caused the greatest nematode mortality, with most deaths occurring within the first hour. Phytochemical analysis identified alkaloids and tannins as the key bioactive compounds responsible for its anthelmintic effects. These findings suggest that betel nut seed extract could be a natural alternative to synthetic dewormers, contributing to sustainable parasite control. However, since this study was conducted only in vitro, further research is necessary to confirm its safety and effectiveness in live animals.

Recommendations

To validate these findings, in vivo studies should be conducted to determine the actual effectiveness of betel nut seed extract in infected animals. Future research should focus on establishing the optimal dosage and treatment regimen to ensure efficacy while minimizing any potential side effects. It is also important to assess its impact on animal health and productivity, as well as its cost-effectiveness compared to commercial anthelmintics. By addressing these aspects, betel nut seed extract could become a viable natural alternative for parasite management in poultry and livestock farming.

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