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Investigation of In Vitro Antioxidant Activity and Teratogenic Effects of Betel Nut (*Areca catechu* L.) in Zebrafish (*Danio rerio*) Embryos

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Abstract

This study characterized the phytochemical composition, evaluated the antioxidant capacity, and assessed the teratogenic effects of crude betel nut (*Areca catechu* L.) extract. The bioactive constituents were extracted using 95% ethanol. Preliminary phytochemical screening identified the presence of alkaloids. Folin-Ciocalteu assay quantified the phenolic content at 617 mg gallic acid equivalent (GAE) per gram of dried extract, reflecting substantial phenolics such as flavonoids, steroids, and tannins known for anthelmintic properties. The extract exhibited strong free radical scavenging against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, with an EC_{50} value of 1.104 ± 0.312 ppm, comparable to ascorbic acid (1.239 ± 0.086 ppm). Zebrafish embryo assays revealed 1.0 g/kg body weight as the safest exposure concentration after 48 hours, whereas higher doses (1.5 and 2.0 g/kg BW) induced toxicity and teratogenicity, likely attributable to alkaloid constituents. This study uniquely integrates phytochemical, antioxidant, and zebrafish embryo toxicity analyses to identify *A. catechu* as a potential natural anthelmintic and antioxidant source for small ruminants, while establishing 1.0 g/kg BW as a safe therapeutic dose for further drug development.

Introduction

The search for safe, natural, and effective plant-derived compounds has gained momentum as resistance to synthetic drugs continues to threaten both human and animal health systems. In the livestock sector, the small ruminant industry remains an economically important enterprise in the country; however, several production challenges hinder its full potential (Orden *et al.*, 2023). Among these constraints, gastrointestinal nematode infections are considered major causes of disease and production losses in sheep, goats, cattle, and horses (Hou *et al.*, 2022). The widespread emergence of anthelmintic resistance (AR), largely due to the repeated use of chemical dewormers, has further aggravated this problem. AR decreases the efficacy of treatments, allowing persistent parasitic infestations that result in reduced growth rate, poor fertility performance, and increased disease susceptibility (Tariq, 2015; Zajac & Garza, 2020). Consequently, AR not only jeopardizes animal health and productivity but also leads to significant economic losses in terms of treatment costs and reduced yield (Charlier *et al.*, 2022). These challenges underscore the urgent need for alternative strategies, particularly those utilizing bioactive compounds from medicinal plants with proven pharmacological and antioxidant potential.

Areca catechu L., commonly referred to as betel nut, is one such plant recognized for its diverse biological activities and traditional therapeutic uses. The seeds of *A.*

catechu are rich in phytochemicals such as alkaloids (arecoline, arecaidine, guvacine), tannins, flavonoids, and polyphenols (Amudhan *et al.*, 2012; Guo *et al.*, 2024). These compounds give *A. catechu* its distinct color and astringent taste, traits that also reflect its broad pharmacological potential.

Biologically, these compounds exhibit a range of therapeutic effects, including anthelmintic, antimicrobial, and anti-inflammatory effects (Murwani *et al.*, 2022; Febriani *et al.*, 2014; Susanti & Prabowo, 2014). Tannins from *A. catechu* have been reported to interfere with nematode physiology, while the alkaloid arecoline demonstrates direct antiparasitic activity (Barbieri *et al.*, 2014). In addition to its anthelmintic potential, *A. catechu* extract exhibits strong antioxidant activity, particularly in scavenging DPPH radicals, which helps maintain oxidative balance and cellular integrity (Sari *et al.*, 2017).

Despite these promising findings, comprehensive studies that simultaneously examine the phytochemical composition, antioxidant capability, and safety profile of *A. catechu* remain limited. Understanding its possible toxicological and teratogenic implications is crucial for its continued use and development as a natural therapeutic or veterinary agent. The zebrafish (*Danio rerio*) embryo model offers an efficient, well-established system to assess developmental toxicity and teratogenic effects of various plant extracts due to its genetic similarity to higher vertebrates and transparent embryogenesis.

Thus, this study was undertaken to (i) identify the phytochemical compounds, particularly alkaloids, present in *A. catechu* through preliminary phytochemical screening, (ii) evaluate its antioxidant properties using the Folin-Ciocalteu method and DPPH free radical scavenging assay, and (iii) determine its toxicity and teratogenic potential using zebrafish embryos as an in vivo bioassay model.

Materials and Methods

Sample Collection and Processing

The sample preparation for the *A. catechu* was adopted from the Yang *et al.* (2023) and Sungpradit *et al.* (2025), with some modifications in the time of oven drying.

About one kilogram of mature *A. catechu* was harvested from the local community of Lagawe, Ifugao (16.8083° N, 121.1939° E), where the fruit is abundant. The fruits were sun-dried for seven (7) days and oven-dried for one hour (60°C) to remove nuts from the husk. The samples were cut into smaller pieces and ground using a Micro Mill (Sungpradit *et al.*, 2025). The prepared ground *A. catechu* was placed inside a clean and dry glass container, labeled and stored in a cool, dry place until further analysis.

Extract Preparation and Determination of Yield

Nut extract preparation was carried out following the procedure of Guo *et al.* (2024) and Bodoira & Maestri (2020) with modifications on the initial drying procedure.

One hundred grams of ground *A. catechu* was macerated in 1000 mL of 95% ethanol, mixed, covered, and kept at room temperature for three (3) days with occasional shaking. After maceration, the extract was filtered, and the residue was rinsed with fresh solvent; the washings were combined with the primary filtrate. The pooled extract was concentrated using a rotary vacuum evaporator at a water bath temperature of 56°C until most of the solvent had evaporated. The concentrated extract was further dried in a hot-air oven at 50°C for 10 hours to remove residual solvent. The dried extract was powdered using a grinder, weighed, and stored in an airtight container. The percent yield of the crude extract was calculated using the formula:

$$\%\ Yield = \frac{mass\ of\ dried\ extract}{mass\ of\ the\ ground\ \ sample} x\ 100 \qquad (1)$$

Physical Test (Color Determination)

The color characteristics of the samples were evaluated using a colorimeter following the CIELAB color space method, as described by Suzuki *et al.* (2024). This system provides an objective and standardized approach for quantifying color differences in biological samples.

The colorimeter was calibrated using standard black and white tiles before measurement. The color of the samples was then determined and expressed in terms of L*, a*, and b* values based on the CIELAB color space. In this system, L* represents lightness on a scale from 0 (black) to 100 (white). The a* coordinate indicates chromaticity along the green-red axis, where negative values correspond to green and positive values to red. The b* coordinate indicates chromaticity along the blue-yellow axis, where negative values correspond to blue and positive values to yellow. These three parameters together provide an objective measure of color and allow for accurate comparison of color differences among samples.

Phytochemical Screening (Presence of Alkaloids)

Table 1. Standard Interpretation Guide for Preliminary Alkaloid Tests (Mayer's and Wagner's Reagents)

Indication	Observable Results	Interpretation
+	Slight turbidity	Presence of primary alkaloids
++	Definite turbidity	Presence of secondary alkaloids
+++	Heavy precipitate	Presence of tertiary alkaloids
-	No precipitate	Absence of alkaloids

Source: Adapted from Gutap et al. (1980)

The preliminary alkaloidal test was employed using the standard method of Gutap *et al.* (1980). An equivalent of 20 grams of crude extract was evaporated to a syrup consistency on an evaporating dish over a steam bath. The concentrated extract was added with 5 mL of 2M HCl, and the mixture was stirred while heated for about 5 minutes, then cooled to room temperature. Five grams of NaCl were added and filtered. The filtrate was diluted to 5 mL with 2M HCl. One test tube was filled with three drops of Mayer's reagent, while the other test tube was filled with Wagner's reagent. The results were observed and recorded, as described in Table 1.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the extract was determined using the Folin-Ciocalteu spectrophotometric method described by Mahdi-pour *et al.* (2012) with some modifications.

Gallic acid was used to construct the calibration curve, with different concentrations prepared (100, 60, 40, 20, and 10 ppm) in ethanol. For the assay, 400 µL of the crude extract was diluted with 800 µL of distilled water. Then, 1 mL of Folin-Ciocalteu reagent was added to the diluted extract. The mixture was shaken vigorously and allowed to stand for 5 minutes at room temperature. Subsequently, 2 mL of 7.5% Na_2CO_3 solution was added. The mixture was shaken thoroughly and allowed to stand in the dark for 90 minutes. Absorbance was measured at 765 nm using a UV-Vis scanning spectrophotometer (T60, PG Instruments Ltd., Leicestershire, UK).

The test was performed in triplicate. The TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram of dried sample, calculated using the following equation:

$$TPC = \frac{concentration\ of\ gallic\ acid\ x\ mass\ of\ crude\ extract}{mass\ of\ dried\ sample} \quad (2)$$

Determination of Antioxidant Activity (DPPH Free Radical Scavenging Assay)

The ability of the *A. catechu* extract to scavenge DPPH free radicals was determined following Lai and Lim (2011); the assay was carried out in methanol but using DPPH (Sigma-Aldrich) at 0.152 mM concentration instead of 0.1 mM.

The EC50 was established using a series of extract concentrations (1000, 300, 100, 10, and 0.1 ppm) and 1.5 mL of each concentration was separately added to 2.5 mL of DPPH solution (6.0 mg DPPH in 100 mL methanol). The mixture was shaken vigorously and left to stand in the dark at room temperature for 30 minutes. The absorbance of the mixture was read at 517 nm using a UV-Vis scanning spectrophotometer (T60, PG Instruments Ltd., Leicestershire, UK). Ascorbic acid (Unilab) was used as a reference standard, and methanol was used as a blank. Triplicated sets of the above concentrations were prepared and used against DPPH free radicals. The percentage of free radical scavenging was calculated as:

$$\%Scavenged = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \quad (3)$$

EC_{50} values were derived from the concentration-response curve obtained by plotting the percentage of DPPH radical scavenging activity against extract concentrations. Non-linear regression analysis (four-parameter logistic model) was performed using GraphPad Prism version 7.0 (GraphPad Prism Software, San Diego, CA, USA).

Determination of Toxicity and Teratogenicity Assay in Zebrafish Embryos

Zebrafish (*Danio rerio*) embryos were obtained following standard spawning and fertilization procedures (Nagel, 2002; Dulay *et al*., 2018) and were used for toxicity and teratogenicity evaluation.

The toxicity and teratogenicity of the compound were evaluated following the protocol described by Dulay *et al.* (2012). In that study, embryos were exposed to increasing concentrations of *Ganoderma lucidum* extract and endpoints such as mortality, hatchability, and morphological malformations were observed over time. To translate possible exposure in goats, the concentrations of *A. catechu* extract were selected corresponding to 1.0 g, 1.5 g, and 2.0 g extract per kg body weight. These doses represent low, moderate, and upper exposure levels for goats and permit observation of dose-response effects (Gupta *et al.*, 2017). This conversion bridges the extract's potential application from laboratory to livestock dosage contexts, ensuring the relevance of toxicity observations to practical veterinary use. Embryos at the segmentation phase were exposed to each dose, in triplicate, and observed microscopically at 12, 24, and 48 hours post-treatment for mortality, teratogenic malformations, and hatchability.

Statistical Analysis

The data from the phytochemical screening and total phenolic content were encoded, analyzed, and graphed using Microsoft Excel 365. Antioxidant properties were analyzed using GraphPad Prism 7 to estimate the sample's median inhibition concentration (IC_{50}). Toxicity

assay results were analyzed using Analysis of Variance (ANOVA) to test for differences in means, followed by Duncan's Multiple Range Test to determine significant differences among groups.

Results and Discussion

Extraction Yield and Physical Properties

Using 95% ethanol as the extraction solvent, 100 grams of ground *A. catechu* produced 27.88 grams of crude extract, corresponding to a yield of 27.88%. The yield reflects the efficiency of ethanol in extracting a wide range of phytochemicals due to its polarity and ability to dissolve both polar and non-polar compounds. This indicates that *A. catechu* possesses a substantial amount of bioactive constituents, supporting its potential as a source of therapeutic and functional compounds.

The crude extract obtained was concentrated and characterized by a reddish-brown coloration, a feature commonly associated with the presence of polyphenolic compounds such as tannins and flavonoids. These compounds are known contributors to the color intensity of plant-derived extracts and are often linked to antioxidants and anti-inflammatory properties. The extract also displayed a shiny surface appearance upon drying, suggesting the presence of alkaloids and phenolic constituents that crystallize and reflect light when concentrated.

In terms of physical characteristics, the extract exhibited a fine, powdery texture, which is advantageous for handling and formulation into various dosage forms such as capsules, tablets, or suspensions. It emitted an astringent and slightly bitter odor—traits characteristic of tannins and alkaloids. The astringency may be attributed to tannins, which precipitate proteins and create a drying sensation, while the bitterness is typical of alkaloid-rich plant materials (Table 2).

These sensory attributes—color, texture, and odor—not only provide preliminary evidence of *A. catechu*'s phytochemical composition but also relate to its longstanding traditional use. The astringent property, for instance, aligns with its application in treating inflammatory and infectious conditions, as tannins promote tissue contraction and reduce exudates.

Overall, the ethanol extraction of *A. catechu* yielded a phytochemically rich crude extract with distinctive physical and organoleptic characteristics. The high extraction yield and the observable sensory properties highlight the plant's biochemical potential for pharmaceutical, nutraceutical, and veterinary applications. Further analytical studies focusing on compound isolation and structure elucidation are warranted to confirm the specific constituents contributing to its antioxidant and anthelmintic activities.

The yield and physical characteristics of the extract thus served as the starting point for the next set of analyses, which explored how its composition relates to its biological activity.

Presence of Alkaloids

Table 2. Alkaloid Test Results for Areca catechu Extract Using Mayer's and Wagner's Reagents

Reagent	Observable Results	Indicator
Mayer's Reagent	(++) Definite turbidity	Presence of alkaloids
Wagner's Reagent	(++) Definite turbidity	Presence of alkaloids

The formation of distinct turbidity upon the addition of Mayer's and Wagner's reagents indicates a positive test for alkaloids (Table 2). This finding reveals that *A. catechu* contains nitrogenous compounds with significant physiological activity in both humans and animals.

Alkaloids in *A. catechu* are bioactive secondary metabolites known for their diverse pharmacological properties, including anti-inflammatory, anticancer, analgesic, local anesthetic, neuropharmacologic, antimicrobial, and antifungal activities (Kurek, 2019). Such a wide spectrum of biological effects underscores the therapeutic potential of *A. catechu*, explaining its extensive application in traditional medicine and modern pharmacological research.

Previous investigations have provided detailed insights into the alkaloid composition of *A. catechu*. According to Meyer Jones *et al.* (1977), the seeds contain total alkaloids comprising approximately 0.2% to 1.7% by weight, with arecoline accounting for 40–85%, arecaidine for 10–40%, and guvacine for 2–30%. Among these, arecoline is identified as the most pharmacologically active and toxicologically significant alkaloid (Chen *et al.*, 2021). Structurally, arecoline is considered a secondary alkaloid because it contains both secondary and tertiary amine functional groups.

While earlier studies focused on quantifying major alkaloids, they did not clearly identify the presence of tertiary alkaloids. The present findings strengthen existing reports by confirming the presence of these compounds through the successful application of Mayer's and Wagner's tests (Table 2). This contributes valuable information to the growing understanding of the complex phytochemical profile of *A. catechu* and enhances its characterization as a rich source of bioactive nitrogenous constituents.

Overall, the confirmation of alkaloids—particularly secondary alkaloids such as arecoline—highlights the pharmacological relevance of *A. catechu*. These compounds play crucial roles in mediating its biological activities and provide a biochemical basis for many of its traditional therapeutic uses. The detected alkaloids, which are known from previous reports to possess various pharmacological activities, emphasize the need for further chemical characterization and toxicity evaluation.

Total Phenolic Content

The total phenolic content (TPC) of *Areca catechu* was found to be 617.73 milligrams gallic acid equivalent (GAE) per gram of dried sample, indicating a remarkably high concentration of phenolic compounds. This substantial TPC value reflects the plant's rich reservoir of natural antioxidants, which play a vital role in counteracting reactive oxygen species (ROS) and preventing oxidative stress–induced cellular damage (Cruz *et al*., 2022). Phenolic compounds act as efficient hydrogen or electron donors, stabilizing free radicals and thereby preserving cellular integrity.

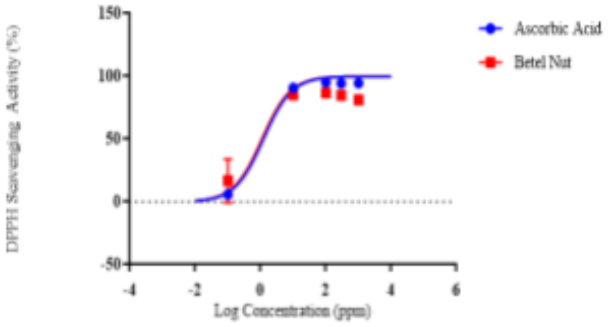

Figure 1. Percentage DPPH radical scavenging activity of the ascorbic acid and *A. catechu* crude extract compared to ascorbic acid

The antioxidant capacity of *A. catechu*, as demonstrated by its effective DPPH radical scavenging activity (Figure 1), can be directly associated with its high phenolic content (Zhang *et al.*, 2014). Studies have consistently shown a positive correlation between TPC and antioxidant activity, as phenolic constituents such as flavonoids, tannins, and steroids readily participate in redox reactions, neutralizing unstable molecules that may cause oxidative deterioration (Mandal *et al.*, 2010; Jeandet, 2015, as cited by Noreen *et al.*, 2017). These antioxidative mechanisms underpin many of the health-promoting properties attributed to *A. catechu*, including potential protective effects against oxidative damage in biological systems.

Beyond antioxidant functions, the phenolic compounds in *A. catechu* are also implicated in its anthelmintic activity. Phenolic compounds such as tannins can interact with anthelmintic targets through hydrogen bonding and hydrophobic interactions, which may disrupt parasite metabolism and neuromuscular activity (Sillanpää, Tähtinen, & Karonen, 2025). The high TPC obtained in this study suggests that the phenolic constituents of *A. catechu* may exert anthelmintic efficacy through both oxidative stress regulation and direct physiological interference with parasites. Additionally, specific phenolic classes, notably tannins and flavonoids, have been documented to inhibit digestive enzymes in parasites and impair nutrient absorption, thereby reducing their viability and reproductive potential (Uniyal *et al.*, 2024).

In summary, the elevated total phenolic content of *A. catechu* reinforces its dual functionality as a strong antioxidant and a promising natural anthelmintic agent. The diverse biochemical roles of its phenolic constituents—ranging from free radical scavenging to antiparasitic action—underscore the plant's pharmacological value and future application potential in promoting animal health, particularly in the development of plant-based therapeutic formulations for small ruminant management.

Antioxidant Potential

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay is a standard and reliable method used to assess the antioxidant potential of natural compounds. This technique measures the ability of antioxidants to donate hydrogen atoms or electrons to the stable DPPH free radical, causing a color change from violet to yellow that indicates scavenging activity (Pavithra & Vadivukkarasi, 2015). In this study, the ethanolic crude extract of *A. catechu* exhibited an absorbance pattern closely aligned with the reference antioxidant, suggesting substantial radical scavenging activity (Figure 1).

Table 3. Median Effective Concentration of Ascorbic Acid and *A. catechu*

Sample	Median Effective Concentration, EC_{50} (ppm)
Ascorbic Acid (Reference)	1.239 ± 0.086[a]
A. catechu Extract	1.104 ± 0.312[a]

The median effective concentration (EC_{50}) of the *A. catechu* extract, as summarized in Table 3, represents the concentration required to neutralize 50% of the DPPH radicals. A lower EC_{50} value corresponds to stronger antioxidant capacity (Cruz *et al.*, 2022). The EC_{50} obtained in this study indicates that the *A. catechu* extract possesses a high level of antioxidant activity comparable to ascorbic acid, a well-known standard antioxidant. Although the EC_{50} value was slightly lower than the 15.95 ± 0.84 ppm reported by Sari *et al.* (2017), this variation can be attributed to

differences in extraction solvents, plant maturity, or assay parameters. Nonetheless, the results affirm the strong radical scavenging potential of *A. catechu* under the conditions used in this study.

The strong antioxidant activity of the extract is largely attributed to its abundant phenolic and flavonoid constituents, as revealed in the phytochemical and total phenolic content analyses. These bioactive compounds are known to quench free radicals, chelate metal ions, and inhibit oxidative chain reactions, thereby preventing lipid peroxidation and cellular damage (Batool *et al.*, 2019, as cited by Ikbal *et al.*, 2020). Flavonoids and polyphenols serve as key antioxidants in medicinal plants by modulating oxidative processes and protecting biological systems from reactive oxygen species (Ferreira-Santos et al., 2021; Zhang *et al.*, 2022).

Overall, the high antioxidant capacity demonstrated by *A. catechu* in the DPPH assay can be directly linked to its rich phenolic profile. This strong free radical scavenging ability not only substantiates its potential as a natural source of antioxidants but also supports its broader pharmacological relevance in mitigating oxidative stress-related conditions and enhancing therapeutic efficacy.

While the antioxidant and phytochemical findings highlight *A. catechu*'s therapeutic potential, its alkaloid content also points to the possibility of toxicity at higher doses.

Effects on the Development of Zebrafish Embryos

Table 4. Toxicity and Teratogenicity of *A. catechu*

Treatment	12 hours	24 hours	48 hours
Control	0.00^{c}	0.00^{b}	0.00^{b}
Treatment 1	0.00^{c}	0.00^{b}	0.00^{b}
Treatment 2	33.33^{b}	77.78^{a}	77.78^{a}
Treatment 3	100.00^{a}	100.00^{a}	100.00^{a}

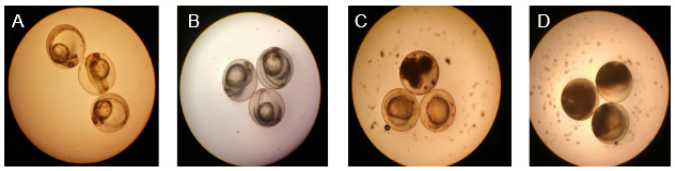

Figure 2. Zebrafish (*Danio rerio*) embryos after 48 hours of exposure to different concentrations of *A. catechu* crude extract. Each panel shows representative embryos under a stereomicroscope: (A) untreated control group with normal morphology; (B) embryos at 1.0 g/kg BW (safe concentration) showing normal development; (C) embryos at 1.5 g/kg BW exhibiting morphological abnormalities and darkened regions indicative of toxicity; and (D) embryos at 2.0 g/kg BW with severe teratogenic effects. Differences in pigmentation and structural integrity highlight the dose-dependent toxicity of the extract.

The toxicity and teratogenicity assay of *A. catechu* extract demonstrated a clear concentration-dependent response in zebrafish embryos. Both the Control and Treatment 1 (1.0 g *A. catechu*/kg body weight) exhibited no mortality within 48 hours post-fertilization, indicating that the lowest tested concentration did not induce toxic or teratogenic effects (Table 4 and Figure 2). This suggests that *A. catechu* at moderate levels may be relatively safe and non-teratogenic under short-term exposure conditions.

In contrast, exposure to higher concentrations resulted in pronounced embryotoxic and teratogenic effects. Treatment 2 (1.5 g/kg) produced a 77.78% mortality rate, while Treatment 3 (2.0 g/kg) resulted in 100% mortality within 48 hours (Table 4). These outcomes indicate that elevated doses of *A. catechu* extract can lead to severe developmental impairments and embryo lethality. The findings are consistent with reported toxicities associated with high consumption of *A. catechu*, emphasizing the potential risks of excessive intake, especially during gestational periods.

The teratogenic and lethal effects observed in zebrafish embryos can be largely attributed to the presence of alkaloids, particularly arecoline, which is recognized as the principal bioactive and toxic compound in *A. catechu*. Arecoline has been reported to produce several adverse outcomes, including teratogenicity, reproductive toxicity, and neurodevelopmental impairment (Chen *et al.*, 2021). The alkaloid content of *A. catechu* typically ranges from 0.3% to 0.7%, with arecoline representing the major fraction. Studies by Peng *et al.* (2015) and Tangalin *et al.* (2011) have shown that exposure to high concentrations of arecoline can disrupt embryogenesis, leading to developmental malformations and growth retardation. Similarly, Siregar *et al.* (2021) and Yan *et al.* (2023) reported that arecoline exposure alters neural development, induces oxidative stress, and causes morphological deformities in zebrafish embryos.

Collectively, the findings of this study corroborate earlier reports on the toxic potential of *A. catechu* at high concentrations. The increased mortality and occurrence of deformities in zebrafish embryos at elevated doses highlight the need for caution in its therapeutic or dietary applications. While *A. catechu* exhibits promising pharmacological properties, its use, particularly among pregnant animals or during critical stages of development, should be carefully regulated to prevent possible teratogenic outcomes, including embryonic deformities or pregnancy loss.

Conclusion

The findings of the study demonstrated that *Areca catechu* possesses a rich phytochemical and bioactive profile with notable antioxidant potential. Preliminary phytochemical screening confirmed the presence of alkaloids, indicated by positive reactions with Mayer's and Wagner's reagents. In addition, *A. catechu* exhibited high levels of phenolic compounds—617.73 milligrams gallic acid equivalent (GAE) per gram of dried sample as determined by the Folin-Ciocalteu method—reflecting its strong capacity to donate electrons and neutralize free radicals. The DPPH free radical scavenging assay further revealed that the antioxidant activity of *A. catechu* is comparable to that of ascorbic acid, emphasizing its potential as a natural antioxidant source.

Toxicity and teratogenicity assays using zebrafish embryos showed that *A. catechu* at a concentration of 1.0 g/kg body weight is relatively safe, causing no mortality or developmental abnormalities within the test duration. However, higher concentrations resulted in increased mortality and teratogenic effects, likely due to the presence of alkaloids such as arecoline. These results suggest that *A. catechu* possesses bioactive constituents with both beneficial and potentially adverse effects, depending on dosage and exposure level.

Overall, *A. catechu* demonstrates significant pharmacological potential owing to its antioxidant and possible anthelmintic properties. The study highlights its viability as a source of natural compounds for therapeutic applications, particularly in animal health management. Further studies are recommended to characterize its bioactive compounds using advanced chromatographic and spectroscopic techniques. Additionally, the exploration of *A. catechu* as a natural anthelmintic agent for small ruminants and its potential integration with nanotechnology-based formulations could pave the way for more effective, safe, and innovative treatment options in veterinary and biomedical research.

Ethical Statement

All experimental procedures involving zebrafish (*Danio rerio*) were approved by the Institutional Animal Care and Use Committee (IACUC) of Central Luzon State University and conducted in accordance with ERC and IACUC ethical standards. Humane care and monitoring were ensured throughout the study by adhering to the principles of the 3Rs (Reduction, Refinement, and Replacement), while confidentiality, integrity, and ethical reporting were maintained during and after the research.

Conflict of Interest Statement

The authors declare no conflict of interest related to the conduct and publication of this research. All procedures followed were in accordance with institutional and ethical standards, and there were no financial or personal relationships that could have influenced the outcomes of this study.

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Declaration of Generative AI and AI-Assisted Technologies

This work was prepared entirely by the author(s) without the use of generative AI or AI-assisted technologies.

Data Availability

All data supporting the findings of this study are available within the paper.

Author Contributions

ESV: Conceptualization, Investigation, Writing (Original Draft), Writing (Review and Editing); **DSP**: Supervision, Resources, Writing (Review and Editing); **NADR**: Writing (Review and Editing); **EAO**: Conceptualization

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References

Amudhan, M. S., Begum, V. H., & Hebbar, K. B. (2012). A review on phytochemical and pharmacological potential of *Areca catechu* L. seed. *International Journal of Pharmaceutical Sciences and Research, 3*(11), 4151–4157.

Barbieri, A. M. E., Fornazari, B. C., Canova, E. B., Moreira, E. L., & Katiki, L. M. (2014). Effectiveness of *Areca catechu* Linn against *Haemonchus contortus* in vitro egg hatch assay. *Boletim de Indústria Animal*, *71*(Suppl).

Bodoira, R., & Maestri, D. (2020). Phenolic compounds from nuts: Extraction, chemical profiles, and bioactivity. *Journal of Agricultural and Food Chemistry*, *68*(4), 927–942. https://doi.org/10.1021/acs.jafc.9b07160

Charlier, J., Bartley, D. J., Sotiraki, S., Martinez-Valladares, M., Claerebout, E., von Samson-Himmelstjerna, G., Thamsborg, S. M., Hoste, H., Morgan, E. R., & Rinaldi, L. (2022). Anthelmintic resistance in ruminants: Challenges and solutions. Advances in Parasitology, 115, 171–227. https://doi.org/10.1016/bs.apar.2021.12.002

Chen, X., He, Y., & Deng, Y. (2021). Chemical composition, pharmacological, and toxicological effects of betel nut. *Evidence-Based Complementary and Alternative Medicine*, *2021*, 1808081. https://doi.org/10.1155/2021/1808081

Cruz, A. D., Feliciano, M. A. M., Paragas, D. S., Detablan, J. A., & Tsai, P. W. (2022). Isolation, characterization, and antioxidant activity of *Selliguea taeniata* secondary metabolites. *Biointerface Research in Applied Chemistry*, *13*, 330.

Dulay, R. M. R., Kalaw, S. P., Reyes, R. G., Alfonso, N. F., & Eguchi, F. (2012). Teratogenic and toxic effects of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Higher Basidiomycetes), on zebrafish embryo as model. *International Journal of Medicinal Mushrooms*, *14*(5), 507–512.

Dulay, R. M., Pamiloza, D. G., & Ramirez, R. L. (2018). Toxic and teratogenic effects of mycelia and fruiting body extracts of *Lentinus strigosus* (BIL 1324) in zebrafish (*Danio rerio*) embryo. *International Journal of Biosciences*, *13*(5), 205–211. http://dx.doi.org/10.12692/ijb/13.5.205-211

Febriani, Y., Hidayat, S., & Seftiana, S. (2014). Anti-worm activity of the areca ethanol extract (*Areca catechu* L.) against *Ascaridia galli* [In Indonesian]. *Indonesian Journal of Pharmaceutical Science and Technology*, *3*(1), 1–7.

Ferreira-Santos, P., Ibarz, R., Fernandes, J. M., Pinheiro, A. C., Botelho, C., Rocha, C. M., ... & Martín-Belloso, O. (2021). Encapsulated pine bark polyphenolic extract during gastrointestinal digestion: Bioaccessibility, bioactivity and oxidative stress prevention. Foods, 10(2), 328. https://doi.org/10.3390/foods10020328

Guo, Z., Wang, Z., Luo, Y., Ma, L., Hu, X., Chen, F., ... & Jia, M. (2024). Extraction and identification of bioactive compounds from areca nut (*Areca catechu* L.) and potential for future applications. *Food Frontiers*. https://doi.org/10.1002/ffi2.443

Gupta, M. K., Rao, M. L. V., Dixit, P., Shukla, P. C., Baghel, R. P. S., & Dixit, A. K. (2017). Anthelmintic activity of a herbal formulation against gastrointestinal nematodes of goats. *Journal of Veterinary Parasitology*, *31*(2), 58–63.

Hou, B., Yong, R., Wuen, J., Zhang, Y., Buyin, B., Subu, D., Zha, H., Li, H. & Hasi, S. (2022). Positivity rate investigation and anthelmintic resistance analysis of gastrointestinal nematodes in sheep and cattle in Ordos, China. *Animals*, *12*(7), 891. https://doi.org/10.3390/ani12070891

Ikbal, A. M. A., Rajkhowa, A., Singh, P. C., Choudhury, P. D., & Sahu, R. K. (2020). Assessment of phytochemical and anthelmintic activity of some selected ethnomedicinal plants from Barak Valley Region of Assam. *Biomedical and Pharmacology Journal*, *13*(4), 1961–1969. https://dx.doi.org/10.13005/bpj/2057

Jeandet, P. (2015). Phytoalexins: Current progress and future prospects. *Molecules*, *20*(2), 2770–2774. https://doi.org/10.3390/molecules20022770

Kurek, J. (2019). Introductory chapter: Alkaloids – Their importance in nature and for human life. In J. Kurek (Ed.), *Alkaloids – Their importance in nature and human life*. IntechOpen. https://doi.org/10.5772/intechopen.85400

Lai, H. Y., & Lim, Y. Y. (2011). Evaluation of antioxidant activities of the methanolic extracts of selected ferns in Malaysia. International Journal of Environmental Science and Development, 2(6), 442–447. https://doi.org/10.7763/IJESD.2011.V2.166

Mahdi-Pour B., Sasidharan S., Sasidharan M., et al. Antioxidant activity of methanol extracts of different parts of Lantana camara. Asian Pac J Trop Biomed. 2012;2(12):960-965. doi:10.1016/S2221-1691(13)60007-6

Mandal, S. M., Chakraborty, D., & Dey, S. (2010). Phenolic acids act as signaling molecules in plant–microbe symbioses. *Plant Signaling & Behavior*, *5*(4), 359–368. https://doi.org/10.4161/psb.5.4.10871

Meyer Jones, L., Booth, N. H., & McDonald, L. E. (1977). *Veterinary pharmacology and therapeutics* (4th ed.). Iowa State University Press.

Murwani, R., Kusumanti, E., & Naumova, E. N. (2022). *Areca catechu* L. and *Anredera cordifolia (Ten) Steenis* supplementation reduces faecal parasites and improves caecal histopathology in laying hens. International Journal of Veterinary Science and Medicine, 10(1), 52–63. https://doi.org/10.1080/23144599.2022.2090732

Nagel, R. (2002). DarT: The embryo test with the zebrafish *Danio rerio*—A general model in ecotoxicology and toxicology. *ALTEX*, *19*(1), 38–48.

Noreen, H., Semmar, N., Farman, M., & McCullagh, J. (2017). Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. *Asian Pacific Journal of Tropical Medicine*, *10*(8), 792–801. https://doi.org/10.1016/j.apjtm.2017.07.024

Orden, E. A., Del Rosario, N. A., Orden, M. E. M., Dela Cruz, J., Veluz, E. S., Crisostomo, J. C. A., & Del Rosario, L. L. M. (2023). Farm characteristics, management practices and production performance of smallholder goat farms in Central Luzon. *Tropical Animal Health and Production*, *19*(4), 1733–1752.

Pavithra, K., & Vadivukkarasi, S. (2015). Evaluation of free radical scavenging activity of various leaf extracts from *Kedrostis foetidissima* (Jacq.) Cogn. *Food Science and Human Wellness*, *4*(2), 81–86. https://doi.org/10.1016/j.fshw.2015.02.001

Peng, W. H., Lee, Y. C., Chau, Y. P., Lu, K. S., & Kung, H. N. (2015). Short-term exposure of zebrafish embryos to arecoline leads to retarded growth, motor impairment, and somite muscle fiber changes. Zebrafish, 12(1), 58-70. https://doi.org/10.1089/zeb.2014.1010

Sari, N., Kuspradini, H., Amirta, R., & Kusuma, I. (2017, November 9). Antioxidant activity of an invasive plant, *Melastoma malabathricum*, and its potential as herbal tea product. In *Proceedings of the IOP Conference Series: Earth and Environmental Science* (Vol. 60, p. 012014). IOP Publishing. https://iopscience.iop.org/article/10.1088/1755-1315/144/1/012029

Siregar, P., Audira, G., Feng, L.-Y., Lee, J.-H., Santoso, F., Yu, W.-H., Lai, Y.-H., Li, J.-H., Lin, Y.-T., Chen, J.-R., & Hsiao, C.-D. (2021). Pharmaceutical assessment suggests locomotion hyperactivity in zebrafish triggered by arecoline might be associated with multiple muscarinic acetylcholine receptors activation. Toxins, 13(4), 259. https://doi.org/10.3390/toxins13040259

Sungpradit, S., Leesombun, A., Chanakarn, C., Nakthong, C., & Boonmasawai, S. (2025). Anthelmintic effects of *Areca catechu* L. (Arecaceae) and *Piper betle* L. (Piperaceae) combination on adult *Haemonchus* spp.: A scanning electron microscopy study. *BMC Veterinary Research*, *21*(1), 491.

https://bmcvetres.biomedcentral.com/articles/10.1186/s12917-025-04951-1

Sillanpää, M., Tahtinen, P., & Karonen, M. (2025). Molecular Aspects of Tannin-Anthelmintic Interactions as Revealed by NMR Spectroscopy. ACS omega, 10(29), 32174-32188. https://doi.org/10.1021/acsomega.5c03937

Susanti, A. E., & Prabowo, A. (2014, September 16). Potency of *Areca catechu* as anthelmintic agent for animals [Conference presentation]. *Prosiding Seminar Nasional Pertanian Ramah Lingkungan Mendukung Bioindustri di Lahan Suboptimal*, Palembang, Indonesia.

Suzuki, T., Ito, C., Kitano, K., & Yamaguchi, T. (2024). CIELAB color space as a field for tracking color-changing chemical reactions of polymeric pH indicators. *ACS Omega*, *9*(34), 36682–36689. https://doi.org/10.1021/acsomega.4c05320

Tariq, K. A. (2015). A review of the epidemiology and control of gastrointestinal nematode infections of small ruminants. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*(3), 693–703. https://link.springer.com/article/10.1007/s40011-014-0385-9

Tangalin, M. G. G. (2011). Anthelmintic effects of processed mature betel nut as dewormer to native chicken and small ruminants (sheep and goats). *Asian Journal of Health Basic Research Section*, *1*, 230–243.

Yan, W., Zhang, T., Li, S., Wang, Y., Zhu, L., Cao, Y., Lai, X., & Huang, H. (2023). Oxidative stress and endoplasmic reticulum stress contributes to arecoline and its secondary metabolites-induced dyskinesia in zebrafish embryos. International Journal of Molecular Sciences, 24(7), 6327. https://doi.org/10.3390/ijms24076327

Yang, B., Chen, H., Chen, W., Chen, W., Zhong, Q., Zhang, M., & Pei, J. (2023). Edible quality analysis of different areca nuts: Compositions, texture characteristics and flavor release behaviors. *Foods*, *12*(9), 1749. https://doi.org/10.3390/foods12091749

Uniyal, S., Chaurasiya, A. K., Chaudhary, P., & Chahal, U. S. (2024). Exploring Condensed Tannin to Control Gastro-Intestinal Parasitism in Small Ruminants. In Feed Additives and Supplements for Ruminants (pp. 235-248). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-97-0794-2_10

Zajac, A. M., & Garza, J. (2020). Biology, epidemiology, and control of gastrointestinal nematodes of small ruminants. *Veterinary Clinics of North America: Food Animal Practice*, *36*(1), 73–87. https://doi.org/10.1016/j.cvfa.2019.12.005

Zhang, W. M., Huang, W. Y., Chen, W. X., Han, L., & Zhang, H. D. (2014). Optimization of extraction conditions of areca seed polyphenols and evaluation of their antioxidant activities. *Molecules*, *19*(10), 16416–16427. https://doi.org/10.3390/molecules191016416

Zhang, P., Sari, E. F., McCullough, M. J., & Cirillo, N. (2022). Metabolomic profile of Indonesian betel quids. *Biomolecules*, *12*(10), 1469. https://doi.org/10.3390/biom12101469